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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> The purpose of this program is to develop early warning disease detection systems for emerging zoonotic diseases in the Asia-Pacific, using the latest technology available, including full length sequencing, deep sequencing, and genomic and proteomic microarrays, and to understand how the evolution of dengue viruses influence epidemic potential. Each day, thousands of people throughout Asia present with illnesses that go undiagnosed. Some of these illnesses will be newly recognized diseases that have epidemic potential such as SARS, Nipah encephalitis, dengue and avian influenza. The tasks described in this proposal will help identify these pathogens before they begin to spread and cause major epidemics. By building on existing relationships and collaborations in Viet Nam, the APITMID will be an important ally to health care and security agencies in preventing the spread of infectious diseases outside of Asia. This has profound and far-reaching implications for global public health and economic security as well as for US homeland defense and military readiness concerns.					
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## INTRODUCTION

Once a major source of human mortality, emerging and re-emerging infectious diseases (EIDs) have again risen to become a leading cause of morbidity and mortality in the New Millennium (Fauci, A.S, Touchette, N.A, Folkers, G.K., 2005. Emerging Infectious Diseases: a 10-Year Perspective From the National Institute of Allergy and Infectious Diseases. *Emerging Infectious Diseases* 11(4):519-525.). In spite of a well-developed economic and medical infrastructure, the US is highly vulnerable to EIDs and exotic infectious pathogens of animals that jump species and emerge in the less-developed areas of Southeast Asia. Epidemics such as pneumonic plague in India in 1994 and the Nipah encephalitis virus outbreak in Malaysia in 1998-1999 threaten to spread beyond borders as finally demonstrated by the outbreak of severe acute respiratory syndrome (SARS) in 2003 caused by a novel zoonotic corona virus. Plague and SARS outbreaks were wake up calls that both known and newly recognized infectious diseases will continue to emerge, and that with today's rapid transportation and globalization, they may threaten not only the public health but also national and international economies.

EIDs have thrived on a combination of demographic, socioeconomic, environmental and ecological global trends that have accelerated over the past few decades, including:

- Unprecedented migration to, and population growth in, the cities of the developing world
- Uncontrolled urbanization
- Other environmental change (e.g., deforestation, land use and agricultural practices)
- Changing animal husbandry practices
- Modern transportation and globalization, with the rapid and massive movement of people, animals and commodities (Reviewed in Gubler, D.J., 2009. Vector-borne diseases. *Rev Sci Tech.* 28(2):583-8.)

These global trends were the principal drivers for the dramatic re-emergence of infectious diseases in the last two decades of the 20th century, and they are projected to continue into the next century (Cohen, M.L. 2000. *Nature* 406:762-767). Many diseases that were effectively controlled in the 1960s (cholera, tuberculosis, typhoid, malaria, dengue/dengue hemorrhagic fever, West Nile fever, yellow fever, Japanese encephalitis, plague, etc.) have re-emerged, causing major epidemics and

increased morbidity and mortality. In addition, and perhaps more alarming, many newly recognized diseases have emerged as major public health problems during this time. These include AIDS, Ebola, Marburg, Lassa, Bolivian, Argentine and Venezuelan hemorrhagic fevers, Nipah encephalitis, SARS, avian influenza and a number of other viral, bacterial and protozoal water-, food-, rodent- and vector-borne infectious diseases. Finally, dramatic advances in biotechnology combined with socio-political issues have greatly increased the risk of bioterrorism. As we enter the 21st century, emerging infectious diseases are one of the most important threats to global public health and economic security.

To address this threat, we proposed to develop early warning disease detection systems for emerging infectious diseases, using advanced technologies to identify newly recognized pathogens. Toward this end, we studied the effect of genetic change on the epidemic potential of dengue viruses, the cause of one of the most important epidemic diseases in Asian and American tropical countries. We also accomplished studies on drugs and vaccines for HIV. The details of our accomplishments according to three main tasks is as follows:

Task 1: Emerging Infectious Diseases: Develop a pilot, zoonotic disease detection program in the Asia Pacific Region.

*Hypothesis: Etiologic agents are present and identifiable with advanced technology for most undiagnosed febrile cases in Southeast Asia.*

Task 2: Emerging Infectious Diseases: Dengue virus (DENV) genetic study.

*Hypothesis: Dengue emergence potential is affected by characteristics of the virus genome that exhibit measurable phenotypic effects in vitro and in mosquitoes.*

Task 3: HIV/AIDS Protocols:

a. Development of Molecular Adjuvants for Therapeutic HIV-1 Vaccines.

*Hypothesis: CD40 immunogenic adjuvant will increase the degree of immune response to HIV-1 vaccine candidate.*

b. Prospective randomized pilot study of the effect of Niaspan ® on endothelial function

in HIV-infected subjects with low HDL cholesterol levels.

*Hypothesis: Short-term Niaspan ® treatment will increase flow-mediated vasodilation of the brachial artery in HIV-infected subjects.*

c. Pilot study identifying acute sero-converters in a High Risk Clinic.

*Hypothesis: Retrospective screening of human samples from a high risk clinic for HIV infection will reveal useful patterns particularly with respect to response to HAART treatment.*

## BODY

This section describes the results of each task made during this project.

Task 1: Develop a pilot zoonotic disease detection program. This task necessitated several logistical and administrative accomplishments to exchange and manage funds and execute the project, as well as institutional protocol approvals involving all three bodies: Vietnam, at the UH and at the U.S. Army Medical Command. This task was accomplished and continues to provide on-going sources of scientific translational collaboration with UH.

T1.1 Technical objective: Develop strong working partnerships with selected institutions in North Vietnam.

We developed a working partnership with the primary collaborating institutions in Vietnam, the National Institute of Hygiene and Epidemiology (NIHE) and the Hanoi School of Public Health (HSPH). This included:

- A seminal trip by Dr. Gubler and team to Vietnam in September 8-18, 2007 to establish sites, meet with key personnel, and train personnel. IRB protocols were finalized and sites were visited, including Bach Mai Hospital, and Army 103 and 108 in Hanoi. Blood bank screening was established. Met with leaders in the Vietnam Health institutions: Professor Truong Uyen NINH, Nguyen Tran Hien (Director), Dr. Le Thi Quynh Mai (Head, Avian Influenza Dept.), Pham Ngoc Dinh (Vice Director) and Thang Truong at National Institute of Hygiene and Epidemiology, NIHE, Bs. Ts. Tran Thuy Hanh, Bach Mai Hospital Director, Le Vu ANH (Dean) at Hanoi School of Public Health, HSPH, Dr. Trinh Quan Huan (Vice

Minister of Health), Dave Dennis (CDC), and Phil Easterman (East-West Center, Hawaii).

- Important field sites were established: Bac Mai Hospital in Hanoi, the largest infectious disease referral hospital in Vietnam, Long Son Provincial Hospital, Viet Tiep City Hospital in Hai Phong, Hoa Binh District Hospital, Cao Bang District Hospital, and My Tho, a city 70 miles south of Ho Chi Minh City, in collaboration with Institute Pasteur in Ho Chi Minh City. These institutions represent both urban and rural sites, including the provinces bordering China.
- IRB approvals were obtained and standard protocols in both English and Vietnamese were written, established and implemented.
- Several training trips by Dr. Thang Truong, NIHE staff scientist, Vietnam to University of Hawaii (Sept 2008) and Dr. Bruce Cropp, microbiologist, from UH to Vietnam (Sept 2009).
- Important joint meetings were held to foster exchange amongst experts from US and Vietnam: **Novartis Institute for Tropical Diseases (NITD) First International Symposium on Dengue in Manila, Philippines, Sept 3 – 6, 2009; International Conference on Emerging Infectious Diseases, including the 4th Asia Dengue Research Network Meeting, Singapore, 8-11 December, 2009. Sponsored by the Duke-NUS Graduate Medical School, the Pediatric Dengue Vaccine Initiative and Novartis Institute for Tropical Diseases.**
- Dr. David Dennis was hired with matching funds from Duke-NUS to take up residence in Hanoi, Vietnam to successfully finalize the project and ensure its continuance in the future, with Dr. Hien as Vietnam PI. .

T1.2 Technical Objective: Develop an active syndromic disease detection system to monitor the pathogens causing disease in humans in both urban and rural Vietnamese environments.

- Sample collection at the surveillance sites covered a full season and targeted febrile patients of unknown etiology. Over 6500 samples were collected. On site (Vietnam) diagnostic testing following our standardized protocols were successfully implemented. 4166 samples were shipped out of Vietnam for further diagnostics and laboratory characterization (T1.3).

- Under the leadership of Dr. David Dennis, we implemented a pilot syndromic surveillance for neurologic manifestations of unknown etiology that is forming the basis of additional projects.

T1.3 Technical objective: Identify pathogens causing illness in humans using advanced technology and determine risk factors for infection of selected agents.

- We established approved molecular characterization methods of samples, including off-site inoculation of unknown, diagnostic samples into mosquitoes and mice in the Insectary and BSL3/ABSL3 facilities, respectively.
- Over 6500 samples from febrile patients in Vietnam and Sri Lanka were collected and diagnostic testing implemented in Vietnam. 500 of these samples were collected from patients with severe viral pneumonia of unknown etiology. Of the total samples, 4166 were then subjected to further testing. Viruses found included dengue virus (DENV) 1, 2, 3, 4, Chikungunya virus, seasonal influenza A H1N1, seasonal H3N2, influenza B, Parainfluenza, metapneumovirus, and Hantaviruses. Of the 6500 samples tested overall, approximately 3100 were confirmed negative according to standard diagnostics as well as by multiplex PCR; Several (11) of the dengue-positive samples were sequenced to confirm circulation patterns.
- 100 unknown samples were inoculated into baby mice. Two had been obtained from patients with fatal hemorrhagic disease, showed signs of infection. These have been passed in another litter of mice, and one killed all pups in the litter; the other caused the mice to be runts, thus confirming a transmissible infectious agent. The mouse brains harvested from these litters were screened by microarray for over 500 human and animal pathogens at the Genome Institute of Singapore, with no hits in spite of good positive controls, suggesting new pathogens and the need for further testing by deep sequencing.
- By far a good number of cases proved to be dengue, although they were cryptic by standard diagnostic protocols. **A highly precise and powerful luminex microsphere bead-based assay** to detect dengue antibodies in acute and convalescent samples was developed in Hawaii. This microsphere-based immunoassay (MIA) was developed and validated on field samples from several populations, showing excellent performance in detection of specific IgG, IgM and non-structural protein NS1 antibodies to dengue.

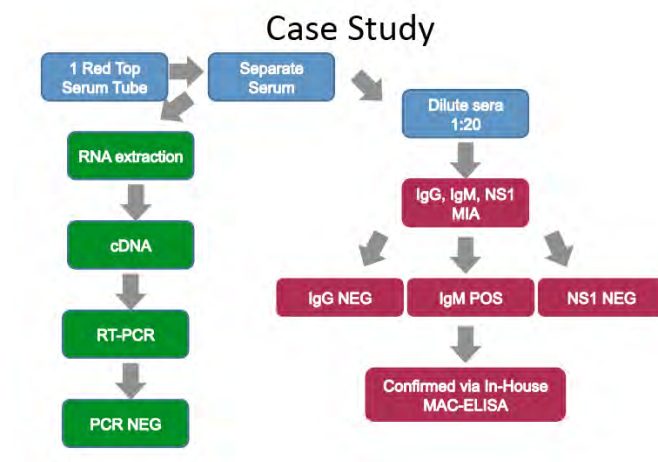


- 292 field samples were validated for IgG reactivity against standard testing methods (PRNTs and the PanBio ELISA) (see Appendix).
- NS1, a highly conserved and secreted glycoprotein, is particularly useful for rapid diagnosis of dengue. A panel of 123 serum samples from Singapore was provided by Duke-NUS to test using the NS1 MIA assay and validated against commercial kits (PanBio Early ELISA, Platelia NS1 assay and NS1 Ag Strip). All samples were previously confirmed to be DENV positive by PCR, DNA sequencing and in most cases virus isolation. The NS1 MIA assay worked well when multiplexed with the IgG and IgM antibody detection assay. There were 17 samples that were correctly diagnosed as DENV positive based on an NS1 positive result when IgM was negative. In addition, our newly developed NS1 assay was able to pick up positive results with early diagnosis for 105/123 (85%) of samples (Appendix).
- **Case study demonstrating the ability of this combined assay approach to correctly diagnose a dengue patient:** We received a call from Kapiolani Medical Center for Women and Children (KMCWC) on Friday June 18, 2010 at 12:00 P.M., regarding a pediatric patient showing dengue-like symptoms. Dengue (DENV) or Chikungunya virus (CHIKV) was suspected. Sampling handling (summarized in figure), and results as follows:
  - The sample was received at 3:00 P.M. After getting 1 red top tube, serum was separated and 140 µL was used for RNA extraction. We isolated RNA, made cDNA, and then ran RT-PCR and results were obtained by **8 P.M.**
  - Concurrently 24 µL of the serum sample was used for MIA assay to test for DENV NS1 antigen, and DENV IgM and IgG antibodies. The sample was processed within 3 hr, with results by **6 P.M.**
  - For confirmatory testing virus isolation was simultaneously set up with results obtained one week later.
  - Our results indicated IgG negative (5 µg/mL IgG) and IgM positive (63.6 ratio), which was further confirmed by our in-house MAC-ELISA (33.7). The sample was PCR negative and virus isolation was negative.

- This sample was originally sent by the clinician to Focus Labs for diagnosis. The first result from Focus Labs was an IgM false negative. After we obtained an IgM positive result, Focus Labs repeated their test and obtained an IgG negative and IgM positive result (IgG IFA 0.35, IgM 5.66) similar to our results.
- We completed standard PRNT at a later date and confirmed that the patient was infected with DENV-2, which concurred with the outbreak in

Bali.

- We received a second sample four days later on June 22, 2010. This sample was IgG low positive and IgM positive.



Task 2: Determine the genetic changes correlated with epidemic patterns and their mechanisms of fixation in a key emerging infectious disease, dengue virus (DENV).

T2.1 The first objective was to determine the historical patterns of genetic variation relative to epidemic behavior in circulation DENV serotypes across multiple populations:

- **South Pacific** isolates of DENV-2 from Tahiti, New Caledonia, American Samoa, and Fiji, from 1971-73 and Tonga 1974, amounting to 21 isolates, were whole-genome sequenced to determine the genetic basis of epidemic attenuation. These viruses caused severe outbreaks across the South Pacific islands in the early 1970s before becoming attenuated in Tonga in 1974, a period of near-silent transmission. Phylogenetic associations indicate that the viruses collected in the South Pacific from 1971 to 1974 were the source of a single introduction from the Caribbean. The attenuated Tongan strain was distinguished from the outbreak strains isolated earlier on the other islands by 2 unique amino acid substitutions in premembrane structural gene and non-structural gene NS4A. A subset was further

distinguished by unique substitutions in NS2A. Manuscript has been published in Virology (Steel et al. 2010)

- **American Samoa** isolates of DENV-4 from a large 2008 outbreak have been sequenced for whole-genome coverage of 1 isolate and complete non-structural gene NS5 coverage of 8 isolates, and analyses suggest a unique genotype has been introduced. Manuscript in prep, Luo et al.
- **Sri Lanka** isolates of DENV-3 from 1982 to 1992 covering a severe epidemic in 1989 which saw the advent of dengue hemorrhagic fever in this long-standing hyperendemic but severe disease-free population: 7 isolates were whole-genome sequenced and indicate unique changes in envelope shared by the 1989 and after severe-disease lineage.
- **Puerto Rico:**
  - DENV-1 isolates from three major outbreaks between 1977 and 1998 have been sequenced for whole-genome coverage of 31 individuals and envelope gene coverage for 18 isolates from the greater Caribbean. Outbreak strains cluster into distinct groups, indicating lineage turnover associated with epidemics. Long divergent branches suggest “evolutionary bursts” underlying outbreak lineages that may indicate selective sweeps. Geographic structure within Puerto Rico appears nonrandom: Ponce emerges as a significantly distinct geographic group, as do the groups San Juan versus non-San Juan, suggesting a role of urban centers such as Ponce and San Juan as foci of transmission. Manuscript in prep, Mueller et al.
  - DENV-2 from the large 1994 outbreak shows a distinct temporal pattern in phylogenetic structure. Monthly isolation data mapped directly on to the phylogeny indicate lineage replacement during the epidemic: from June to August one clade dominated, and from September to December another, distinct clade dominated. Interestingly, the earlier clade persisted into future years. Manuscript in prep, Christenbury et al.
  - DENV-3 isolates, including from around the Caribbean: spanning two decades have been sequenced for 13 isolates for structural gene coverage. This data set has allowed us to demonstrate the phylogeographic movement of viruses amongst countries, particularly between mainlands and islands, and vica versa. Manuscript

in prep, Allicock et al.

- DENV-4 from 1985 to 1995 have been sequenced for 75% coverage of 6 isolates, combined analytically with earlier findings to indicate that epidemic cycles are leaving a similar pattern in the genome evolution. Manuscript published (Bennett et al. 2010 MBE).

T2.2 The second objective was to assess the relative importance of positive selection vs. random genetic drift in the ongoing fixation of genetic variation in DENV, and make phenotypic inferences based on these substitutions.

- **South Pacific:** Selection analyses on the South Pacific isolates do not indicate that positive selection has acted to attenuate the Tongan DENV-2 strains. However, virus demographics that echo epidemiologic data except for an unsuspected increase in the Tongan outbreak, suggesting higher asymptomatic rates underlie epidemic attenuation rather than reduced transmission (Steel et al 2010).
- A strong statistical signal for selection was not found for either the **American Samoa** 2008 outbreak or the **Sri Lanka** 1989 outbreak. However, tests for selection are low-powered and do not test for selective sweeps (single, strong fixation events in the evolutionary history of a taxon).
- **Puerto Rico:**
  - DENV-2 epidemic of 1994 with-epidemic lineage replacement was confirmed by BaTS (<http://evolve.zps.ox.ac.uk/evolve/BaTS.html>).
  - DENV-1 phylogeographic structure, in which urban centers seem to provide foci of infection, was also confirmed using BaTS.
  - DENV-4 sequence data recapitulates demographic changes, showing the importance of true genetic bottlenecks in shaping lineage turnover by a combination of genetic drift and natural selection (Bennett et al 2010).
  - DENV-3: Analyses for demographic changes of DENV of all serotypes in Puerto Rico and the surrounding region shows a general decline in DENV2 presumably in response to the introduction of DENV3. Manuscript in preparation (Allicock et al).
- Several interesting amino acid mutations that distinguish epidemic strains of dengue

viruses were tested for phenotypic effects by infecting with local DENV strains local dengue vector *Aedes albopictus*, taking advantage of our fully functional insectary established at the University of Hawaii, Manoa, JA Burns School of Medicine. Over 5900 mosquitoes were exposed to infectious blood meal, with an average female engorgement rate of 85%. Final analyses ongoing.

Task 3. HIV/AIDS Protocols: Adjuvant development; pilot study on therapeutic Niaspan ®; identification of acute sero-converters.

T3.1 The first objective was development of molecular adjuvants for therapeutic HIV-1 vaccines.

We developed a recombinant ALVAC virus expressing mouse CD40L and co-immunized normal BALB/c mice with this and vCP1452, an ALVAC-based HIV-1 vaccine candidate. Mice were immunized three times at 2-week intervals by injecting 100 µl solution containing 10<sup>7</sup> pfu vCP1452 and 10<sup>7</sup> pfu CD40L-expressing ALVAC or 10<sup>7</sup> pfu parental ALVAC into the quadriceps of both hind limbs (50 µl/each). Parental ALVAC was as a negative control. Six weeks after the last immunization, mice were euthanized and spleens were removed and processed into single-cell suspension in complete RPMI-1640 medium. HIV-1-specific CTLs have been analyzed. Co-immunization of mice with CD40L-expressing ALVAC and the ALVAC-based vaccine expressing HIV-1 proteins, vCP1452, augmented HIV-1 specific cytotoxic T lymphocyte (CTL) responses in terms of frequency, polyfunctionality and interleukin (IL)-7 receptor alpha chain (IL-7Ralpha, CD127) expression. In addition, CD40L expressed from ALVAC virus could significantly augment CD4<sup>+</sup> T cell responses against HIV-1 in mice. CD40L expressed from ALVAC matured human monocyte-derived dendritic cells (MDDCs) in a tumor necrosis factor-alpha (TNF-alpha) independent manner, which underwent less apoptosis, and could expand ex vivo Epstein-Barr virus (EBV)-specific CTL responses from healthy human individuals and ex vivo HIV-1-specific CTL responses from HIV-1-infected individuals in the presence or absence of CD4<sup>+</sup> T cells. Taken together, our results suggest that CD40L incorporation into ALVAC vectors could be used as a strategy to enhance HIV-1 vaccine immunogenicity.

T3.2 The second objective was to conduct a prospective randomized pilot study of the effect of Niaspan ® on endothelial function in HIV.

- This was a randomized controlled study evaluating endothelial function, measured by flow-mediated vasodilation (FMD) of the brachial artery, among HIV-1 infected individuals with low high density lipoprotein (HDL) before and after a 12 week treatment with extended-release niacin. Subjects randomized to the treatment arm received extended-release niacin (Niaspan ®) starting at 500 mg per night and titrated to a maximum tolerated dose (not exceeding 1500 mg per night). Subject in the control arm received the same follow-up as the treatment arm but were not given extended-release niacin and were instructed to not take any supplemental niacin. FMD, lipid parameters, insulin sensitivity and C-reactive protein (CRP) were all obtained at baseline and after week 12 for both control and treatment arms.
- Subjects were eligible for the study if they were HIV infected and 18 years of age or over. Subjects must have been on stable highly active antiretroviral therapy (HAART) 6 months prior to study entry with an HDL less than 40 mg/dL and LDL less than 130 mg/dL. Subjects were excluded if they had cardiac disease, uncontrolled hypertension, pregnancy, and diabetes mellitus. Subjects were ineligible if they were on medication known to influence vasodilatation such as nitrates, metformin, pioglitazone, and rosiglitazone. Treatment with lipid-lowering drugs within 6 weeks prior to study was also not allowed. Subjects who regularly use tobacco products were asked to refrain from using these products for at least 8 and preferably 12 hours prior to the FMD procedure.
- Endothelial function was assessed by FMD. A 12 lead electrocardiogram was monitored throughout the study. Blood pressure was measured with an oscillometric sphygmomanometer in the left upper arm prior to the first scan, prior to the administration of sublingual nitroglycerin, and then every 5 minutes until it returned to baseline. Each study was recorded digitally and sent to the core ultrasound laboratory at the Department of Cardiovascular Medicine in the University of Wisconsin at Madison. Brachial artery diameters were measured in triplicate with a digital border tracing tool (Access Point, Freeland Systems, Westfield, Indiana). Data obtained from FMD, namely, base mean diameter of the brachial artery, maximum FMD, and nitroglycerine FMD

(NTGMD) were compared with baseline characteristics and laboratory data given niacin treatment. Lipid parameters, C-Reactive protein (CRP), CD4, HIV viral load, and insulin sensitivity were obtained on all subjects. A paired t-test was used to determine differences between the control and intervention groups. A two sided probability of  $p < 0.05$  was used to determine statistical significance. All statistical analyses were performed using the JMP statistical program.

- 87 individuals were screened of which 19 subjects were enrolled and completed their study requirements by July, 2009, which included 2 sonograms each for a total of 38 sonographs. Of the 19 subjects there were 17 males and 2 females, with a median age of 50 years (age range 28-65). Four were current smokers and 7 were former smokers. By history, 5 had dyslipidemia (all diet controlled), 3 had a diagnosis of hypertension (all treated with anti-hypertensive medications). The median CD4 count was 493 cells/mm<sup>3</sup> (range 280-1096). All subjects had undetectable HIV viral loads except for 1 subject with a viral load of 1,520 copies/mm<sup>3</sup>. The median CRP level was 0.8 mg/l with a range of <0.2 to 6.3. Lipid parameters showed a median HDL of 34.0 mg/dL (range 19 to 46), median LDL of 115 mg/dL (range 59-165). The median body mass index (BMI) was 25.0 (range 20.2 to 35.8). The majority of subjects were on an efavirenz based regimen (47%), while 42% were on a protease inhibitor (PI) based regimen. Framingham risk scores ranged from <1% to 16% with a median of 7%.
- The median base mean diameter was 0.4500 with a range of 0.3230 and 0.5670. Median value of FMD was 4.42%. In comparison to the non-HIV infected population, a FMD of 7 to 10% from the baseline diameter is considered normal (6-10). Eleven subjects had FMD values below 5%. Older age, smoking, higher BMI, CRP, protease-inhibitor use, lower HDL levels were not associated with lower values for FMD or NTGMD.
- 69% of subjects had decreased endothelial measurements, consistent with levels associated with cardiovascular disease.
- **Effect of Extended Release Niacin (ERN) on FMD:** Niacin treatment demonstrated improvement in FMD
- Participants receiving ERN had a median HDL-C (IQR) increase of 3.0 mg/dL (0.75, 5.0) compared to -1.0 mg/dL in controls (-6.0, 2.5),  $p = 0.04$ .

- End of study FMD for ERN was significantly different from controls after adjusting for baseline differences in FMD and HDL-C, 6.36% (95% CI: 4.85, 7.87) and 2.73% (95% CI: 0.95, 4.51) respectively,  $p=0.048$  (Chow et al. 2010).

### T3.3 The third objective was to conduct a pilot study Identifying Acute Sero-converters in a High Risk Clinic.

We took advantage of stored, previously collected blood samples from a high risk clinic in Thailand to identify acute sero-converters to HIV. Below are the significant results.

- 6426 stored samples at the Thai Red Cross Anonymous Clinic between March 2006 and Sept. 2007 were screened for acute HIV infection by:
  - pooled nucleic acid testing (NAT) of 4th-generation enzyme immunoassay (EIA): 5402 samples were negative, 1024 samples were positive.
  - subsequent 1st generation EIA testing of 4th generation EIA positive samples (n=1024).
  -
- Eleven acute HIV infected subjects were identified by pooled NAT (n=7) and serial EIA (n=4).
  - Mean age 28 years; 9 were male; and 60% were men who have sex with men (MSM).
  - Median HIV RNA was 99,601 copies/ml ( $\log_{10} VL = 5.00$ ).
  - Eight samples could be genotyped: 6 CRF01\_AE, 1 subtype B, 1 CRF01\_AE/B recombinant.
  - No resistance to antiretroviral therapy was found.
- Thus acute HIV infection can be identified using pooled NAT and sequential EIA in a Thai high risk cohort with a prevalence of 20.3 per 10,000 persons at risk (95% CI, 10.1-36.4).
- This gave us an estimated HIV incidence of 2.7 per 100 person-years (95% CI, 2.2-4.3). Ananworanich et al. 2008. J Acquir Immune Defic Syndr. 2008 Oct 1;49(2):151-5.

## KEY RESEARCH ACCOMPLISHMENTS

The following bulletized list of accomplishments offer a quick look at what was gained as a result



of this study:

#### Task 1

- A major collaborative research endeavor between Vietnam and the United States was established and carried out to conduct ongoing country-wide syndromic surveillance of febrile cases of unknown causes in a rigorous manner, such that data could be used to study patterns of emerging infectious diseases. This collaborative project involved extensive training and development of local infrastructure and expertise. Over 6500 samples were screened, involving standard and new technologies. One of these technologies, the microsphere immunoassay (MIA), was developed in Hawaii as a powerful diagnostic tool tested on many of these samples. 3100 samples were confirmed negative. Of the confirmed positives dengue virus (DENV) 1, 2, 3, 4, Chikungunya virus, seasonal influenza A H1N1, seasonal H3N2, influenza B, Parainfluenza, metapneumovirus, and Hantaviruses were found. In addition, several unknown pathogens are suspected and are undergoing testing. This project has successfully established a high quality febrile illness surveillance system by partnering with local officials and expertise, in a country that could represent a major source of infectious disease emergence in the US.

#### Task 2

- Dengue viruses are one of the important emerging infectious diseases plaguing Vietnam and Southeast Asia in general, and also, more recently, the US. Our comparative approach to understand the evolutionary drivers of emergent epidemic strains has revealed that changes in nonstructural genes NS2A, NS4A, and structural gene prM, are potentially critical. In addition, signatures of adaptive change that are correlated with disease severity include accelerated rates suggestive of strong natural selection. Testing emergent strains in mosquitoes promises to indicate the exact mechanisms of selection driving these nonrandom emergent strains.

#### Task 3

- The development of a molecular adjuvant (recombinant ALVAC virus expressing mouse CD40L) for therapeutic HIV-1 vaccines was successful, boosting immunogenicity when used in BALB/c mice co-immunized vCP1452, an ALVAC-based HIV-1 vaccine candidate. The adjuvant augmented HIV-1 specific cytotoxic T lymphocyte (CTL) responses in mice and increased IL-7 receptor expression that could similarly augment

CD4+ T cell responses to infection. In vitro, CD40L expressed from ALVAC in matured human monocyte-derived dendritic cells in a tumor necrosis factor-alpha (TNF-alpha) independent manner, underwent less apoptosis, and could expand ex vivo Epstein-Barr virus (EBV)-specific CTL responses from healthy human individuals and ex vivo HIV-1-specific CTL responses from HIV-1-infected individuals in the presence or absence of CD4+ T cells. Together, these results show that CD40L incorporation into ALVAC vectors could be used as an adjuvant to enhance HIV-1 vaccine immunogenicity (Liu et al. 2008. *Vaccine* 26:4062-72).

- The pilot study on the effect of extended release Niaspan® on endothelial function in HIV-infected subjects demonstrated that endothelial dysfunction is common in HIV infected patients. Sixty nine percent of subjects had decreased endothelial measurements, consistent with levels associated with cardiovascular disease. The subjects had no underlying cardiac history and relatively low risk factors for CAD. We showed that the short-term use of extended release niacin significantly improves flow-mediated vasodilation (FMD) and hence endothelial function of the brachial artery (Chow et al. 2010). These data provided the basis of an NHLBI R21 grant submission under Dr. Chow in January 2010 to study Autonomic Function and Cardiovascular Disease in HIV Infection.
- The pilot study from a high risk HIV clinic in Thailand found that acute HIV infection can be identified using pooled NAT and sequential EIA in a Thai high risk cohort with a prevalence of 20.3 per 10,000 persons at risk (95% CI, 10.1-36.4), and an estimated HIV incidence of 2.7 per 100 person-years (95% CI, 2.2-4.3). Ananworanich et al. 2008. *J Acquir Immune Defic Syndr.* 2008 Oct 1;49(2):151-5. This project was leveraged into additional funding, including a NeuroAIDS R21.

## REPORTABLE OUTCOMES

- Protocols, IRBs were established and approved in Vietnam and the US for syndromic disease surveillance. Research infrastructure and training were put in place.
- A new diagnostic assay for dengue was established with improved sensitivity and specificity that should increase the diagnostic window and improve health outcomes by enabling more accurate and timely treatment.

- Over 6500 cases were screened. Several pathogens were identified, including over 40 whole genomes interrogated.
- An indicator of emergent strains of dengue viruses were identified as those exhibiting accelerated rates of genetic change at the amino acid level relative to neutrality.
- An insectary was established to identify emergent phenotypes.
- A molecular adjuvant was developed and tested in mice that boost immunogenicity of HIV-1 vaccine candidates.
- A new treatment, extended release niacin, for endothelial dysfunction in HIV-1 patients was tested and found effective.
- High throughput screening methods based on pooling samples, for HIV-1 screening, were tested and found to be effective at a high-risk clinic.
- Other reportable outcomes include the following Publications and meetings:

#### Publications:

Ananworanich J, Phanuphak N, de Souza M, Paris R, Arroyo M, Trichavaroj R, Sirivichayakul S, Shikuma C, Phanuphak P, Kim JH; South East Asia Research Collaboration with Hawaii 004 Protocol Team. 2008. Incidence and characterization of acute HIV-1 infection in a high-risk Thai population. *J Acquir Immune Defic Syndr*. 2008 Oct 1;49(2):151-5.

Bennett, S. N., Drummond, A., Kapan, D. D., Suchard, M., Pybus, O., Holmes, E. C., Gubler, D. J. 2010. Epidemic dynamics revealed in dengue evolution. *Mol Biol Evol*. 27:811-818. PMID: 19965886

Chow DC, Stein JH, Seto TB, Mitchell C, Sriratanaviriyakul N, Grandinetti A, Gerschenson M, Shiramizu B, Souza S, Shikuma C. 2010. Short-term effects of extended-release niacin on endothelial function in HIV-infected patients on stable antiretroviral therapy. *AIDS*. 2010 Apr 24;24(7):1019-23. PMID: 20216298.

Chow, DC, Anne Tasaki, Jill Ono, Bruce Shiramizu, Scott A Souza. Effect of Extended-Release Niacin on Hormone-Sensitive Lipase and Lipoprotein Lipase in Patients with HIV-Associated Lipodystrophy Syndrome" *Biologics: Targets & Therapy* 2008;2(4): 1-5.  
[http://www.dovepress.com/articles.php?article\\_id=2586](http://www.dovepress.com/articles.php?article_id=2586)

Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vázquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo Lic NR, Besoff KE, Kliks S, Peeling RW. 2009. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerg Infect Dis*. 15(3):436-40.

Liu J, Yu Q, Stone GW, Yue FY, Ngai N, Jones RB, Kornbluth RS, Ostrowski MA. 2008. CD40L expressed from the canarypox vector, ALVAC, can boost immunogenicity of HIV-1 canarypox vaccine in mice and enhance the in vitro expansion of viral specific CD8(+) T cell memory responses from HIV-1-infected and HIV-1-uninfected individuals. *Vaccine* 26(Jul; 32):4062-72.

Liu J, Ngai N, Stone GW, Yue FY, Ostrowski MA. 2009. The adjuvancy of OX40 ligand (CD252) on an HIV-1 canarypox vaccine. *Vaccine*. 2009 Aug 13;27(37):5077-84. Epub 2009 Jun 30.

- Ooi EE, Gubler DJ. 2009. Dengue in Southeast Asia: epidemiological characteristics and strategic challenges in disease prevention. *Cad Saude Publica*. 25 Suppl 1:S115-24. Review.
- Ooi EE, Wilder-Smith A, Ng LC, Gubler DJ. The 2007 dengue outbreak in Singapore. *Epidemiol Infect*. 2010 Jul; 138: 958-9.
- Steel, A., D.J. Gubler, S.N. Bennett. 2010. Natural attenuation of Dengue Virus Type-2 after a series of island outbreaks: a retrospective phylogenetic study of events in the South Pacific three decades ago. *Virology* 405(2):505-512.
- Wilder-Smith A, Earnest A, Tan SB, Ooi EE, Gubler DJ. 2010. Lack of association of dengue activity with haze. *Epidemiol Infect*. 2010 Jul; 138: 962-7.
- Wilder-Smith A, Gubler DJ. 2008. Geographic expansion of dengue: the impact of international travel. *Med Clin North Am*. 92(Nov; 6):1377-90. Review.
- Wilder-Smith A, Ooi EE, Vasudevan SG, Gubler GJ. 2010. Update on Dengue: Epidemiology, Virus Evolution, Antiviral drugs and Vaccine Development. *Curr Infect. Dis Rep*. 2010 12: 157 - 164.
- Yoksan, Sutee, and Duane J Gubler. 2010. Dengue Vaccine Development: The role of the WHO South-East Asia Regional Office. World Health Organization, Regional Office for South-East Asia. 2010; Document SEA-DEN-9.

#### Book Chapters

- Bennett, S. N. 2010. Evolutionary Dynamics of Dengue Virus. In *Frontiers in Dengue Virus Research*, eds. K. A. Hanley and S.C. Weaver. p. 157-172.
- Gubler, D.J. Urbanization and the Social Ecology of Emerging Infectious Diseases. In *The Social Ecology of Infectious Diseases*. Mayer, KH & Pizer, HF, editors. Academic Press (Elsevier, Inc.), London, 2008. Chapter 4.
- Gubler, D.J. The Global Threat of Emergent/Reemergent Vector-Borne Diseases. In *Vector- Borne Diseases; Understanding the Environmental, Human Health, and Ecological Connections*. Workshop Summary. Institute of Medicine of the National Academics, The National Academies Press, Washington D.C., 2008. pp. 43-64.
- Gubler, D.J. The 20th Century Re-Emergence of Arboviral Diseases: Lessons Learned and Prospects for the Future. In *Arthropod Borne Viral Infections Current Status and Research*. Raghunath, D.; Durga R.C. editors. Tata McGraw-Hill Publishing Co. Limited, New Delhi, 2008. pp. 19-37.
- Ooi, E.-E, Gubler, DJ. 2010. Dengue Virus-Mosquito Interactions. In *Frontiers in Dengue Virus Research*, eds. K. A. Hanley and S.C. Weaver. p. 143-155.
- Peterson LR & Gubler DJ, 2010. Flaviviruses. In Warrell D. (Ed.); *Oxford Textbook of Medicine*, 5th edition, Oxford University Press.

#### Meetings:

- Bennett SN, Akins C, Duran A, Steel A, Cropp CB, Nerurkar VR, Gubler DJ. Evolution of Emergent Dengue Virus: Viral Genotypic Effects on Epidemic Potential. NIAID Regional Center of Excellence Fourth Annual Meeting, St. Louis, MO, April 15-17, 2007.
- Bennett, SN, D. Kapan, A. Drummond, O. Pybus, E. Holmes. Epidemic dynamics revealed in dengue sequence evolution. NIAID Regional Center of Excellence Fifth Annual Meeting, Chicago, IL, April 6-8, 2008.
- Bennett, SN, D. Kapan, A. Drummond, O. Pybus, D. J. Gubler, E. Holmes. Epidemic dynamics revealed in dengue evolution Keystone Symposium, Molecular Evolution as a Driving Force in Infectious Diseases. Breckenridge, CO, April 8-13, 2008.

Bennett, S.N., D.D. Kapan, A. Drummond, O. Pybus, E. Holmes. Epidemic dynamics revealed in dengue sequence evolution. Keystone Symposium, Molecular Evolution as a Driving Force in Infectious Diseases. RCMi Dec 1- 5, 2008, Honolulu HI; MEEGID IX, Oct 29-Nov 1, 2008, Irvine CA

Bennett, S.N., Drummond, A., Kapan, D.D., Suchard, M., Pybus, O., Holmes, E.C., Gubler, D.J. Epidemic dynamics revealed in dengue evolution. American Society of Tropical Medicine and Hygiene, Washington, DC., November 18-22, 2009

Bennett, S.N., Drummond, A., Kapan, D.D., Suchard, M., Pybus, O., Holmes, E.C., Gubler, D.J. Epidemic dynamics revealed in dengue evolution. International Conference on Emerging Infectious Diseases, including the 4th Asia Dengue Research Network Meeting, Singapore, 8-11 December, 2009. Sponsored by the Duke-NUS Graduate Medical School, the Pediatric Dengue Vaccine Initiative and Novartis Institute for Tropical Diseases.

Bennett, S. N., Drummond, A., Kapan, D. D., Munoz, J., Pybus, O., Holmes, E. C. Gubler, D. J. Epidemic dynamics revealed in dengue sequence evolution. Evolution 2010 Meeting, Portland, OR, June 25-29, 2010.

Bennett, S.N. Evolutionary dynamics of dengue viruses: How drift and selection conspire to generate emergent strains. 12<sup>th</sup> Research Centers in Minority Institutions International Symposium on Health Disparities, Nashville, TN, December 6-9, 2010.

Hu. N., Shikuma C., Shiramizu B., Yewdell J., Moss B., Yu Q. HIV Protease Inhibitors Suppress Antigen Expression by Live Poxvirus-based Vaccines: A Potentially Negative Impact of Antiretroviral Therapy on Efficacy of Live Vectored Vaccines. An abstract submitted to 15th Conference on Retroviruses and Opportunistic Infections. February 3-6, 2008, Boston, Massachusetts.

Kaufusi, PH, ML Chapagain, N Altan-Bonnet, VR Nerurkar. WNV NS4B modulates viral RNA replication by remodeling ER membrane. 12<sup>th</sup> Research Centers in Minority Institutions International Symposium on Health Disparities, Nashville, TN, December 6-9, 2010.

Mueller, B. A., Durrell D. D. Kapan, Bruce A. Wilcox, and Shannon N. Bennett. Using weather variation to predict dengue fever in Puerto Rico. EcoHealth, Dec 1 – 5, 2008, Merida, Mexico.

Mueller, B. A., Jorge L. Munoz-Jordan, Duane J. Gubler, & Shannon N. Bennett. Comparative Molecular Evolution of Dengue in Puerto Rico. RCMi Dec 1- 5, 2008, Honolulu HI; MEEGID IX, Oct 29-Nov 1, 2008, Irvine CA; Second International Conference on Dengue and Dengue Hemorrhagic Fever. October 15-17, 2008, Phuket, Thailand. STUDENT TRAVEL AWARD, AWARD FOR BEST STUDENT POSTER PRESENTATION

Mueller, B.A., Paidi, M., Allicock, O., Carrington, C.V., Gubler, D.J., Bennett, S.N. Phylogeography and Molecular Evolution of Dengue Virus Type 1 in Puerto Rico, 1981-1998. American Society of Tropical Medicine and Hygiene, Washington, DC., November 18-22, 2009

Mueller, B.A., Paidi, M., Allicock, O., Carrington, C.V., Gubler, D.J., Bennett, S.N. Phylogeography and Molecular Evolution of Dengue Virus Type 1 in Puerto Rico, 1981-1998. J. A. Burns School of Medicine Biomedical Symposium, Honolulu, HI, April 13, 2010.

Steel, A., Duane J. Gubler, Shannon N. Bennett. Whole-genome Phylogenetic Analysis of Outbreaks of Dengue Type-2 in the South Pacific. RCMi Dec 1- 5, 2008, Honolulu HI; Second International Conference on

Dengue and Dengue Hemorrhagic Fever. October 15-17, 2008, Phuket, Thailand. STUDENT TRAVEL AWARD

Steel, A., Gubler, D.J., Bennett, S.N. Natural Attenuation in a South Pacific Outbreak of Dengue Type-2. American Society of Tropical Medicine and Hygiene, Washington, DC., November 18-22, 2009

Steel, A., Gubler, D.J., Bennett, S.N. Natural Attenuation of Dengue Type-2 Virus During Epidemics in the South Pacific. International Conference on Emerging Infectious Diseases, including the 4th Asia Dengue Research Network Meeting, Singapore, 8-11 December, 2009. Sponsored by the Duke-NUS Graduate Medical School, the Pediatric Dengue Vaccine Initiative and Novartis Institute for Tropical Diseases.

Steel, A., Gubler, D.J., Bennett, S.N. Natural Attenuation of Dengue Type-2 After a Series of Island Outbreaks. J. A. Burns School of Medicine Biomedical Symposium, Honolulu, HI, April 13, 2010.

Volper, EM, H Luo, J Meeks, B Cropp, A Imrie, DJ Gubler, VR Nerurkar: "Rapid Multiplex Microbead Suspension Immunoassay For Measurement of Anti-Dengue Virus Antibodies". American Society for Tropical Medicine and Hygiene. 57<sup>th</sup> annual Conference, Sheraton New Orleans, New Orleans, LA, December 7-11, 2008.

Volper, EM, H Luo, J Meeks, B Cropp, A Imrie, DJ Gubler, VR Nerurkar: "Rapid Multiplex Microbead Suspension Immunoassay For Measurement of Anti-Dengue Virus Antibodies". 11<sup>th</sup> Research Centers in Minority Institutions International Symposium on Health Disparities, Sheraton-Waikiki Hotel, Honolulu, HI, USA, December 1-4, 2008.

Volper, EM, H Luo, B Cropp, J Meeks, A Imrie, DJ Gubler, VR Nerurkar: "Rapid Multiplex Microsphere-Bead Based Immunoassay For Measurement of Anti-Dengue Virus" (poster presentation). American Society for Virology 28th annual Conference, July 11-15, 2009, University of British Columbia, Vancouver, Canada.

Volper, EM, H Olekszak, B Cropp, J Meeks, AJ Johnson, A Imrie, DJ Gubler, VR Nerurkar: "Validation of A Multiplex Microsphere-Based Immunoassay For Measurement of Anti-Dengue Virus Immunoglobulin Antibodies" (poster presentation). American Society of Tropical Medicine and Hygiene 58<sup>th</sup> annual Conference, November 18-22, 2009, Washington D.C, USA.

Volper, EM H Olekszak, B Cropp, J Meeks, AJ Johnson, A Imrie, DJ Gubler, VR Nerurkar: "Validation of A Multiplex Microsphere-Based Immunoassay For Measurement of Anti-Dengue Virus Immunoglobulin Antibodies" (poster presentation). 4<sup>th</sup> Asian Dengue Research Network Meeting, December 8-11, 2009, Duke-NUS, Singapore.

Volper, EM H Olekszak, B Cropp, J Meeks, AJ Johnson, A Imrie, DJ Gubler, VR Nerurkar: "Validation of A Multiplex Microsphere-Based Immunoassay For Measurement of Anti-Dengue Virus Immunoglobulin Antibodies" (poster presentation). JABSOM Biomedical Sciences Symposium, April 13, 2010, Honolulu, HI.

Volper, EM H Olekszak, B Cropp, J Meeks, AJ Johnson, A Imrie, DJ Gubler, VR Nerurkar: "Validation of A Multiplex Microsphere-Based Immunoassay For Measurement of Anti-Dengue Virus Immunoglobulin Antibodies". American Society of Microbiology, April 17, 2010, Honolulu, HI.

White WA, Akins C, Steel A, Bennett SN. The Molecular Basis for Epidemic Variation of Dengue-2 in the South Pacific. J. A. Burns School of Medicine INBRE Program, Honolulu, HI, July 2007.

Yu Q, An invited talk: Strategies for helping HIV-1-specific cytotoxic T lymphocytes: towards development of molecular adjuvants for HIV-1 vaccines. 2008 AIDS & STD Research Symposium, November 21, 2008. University of Washington, Seattle, WA.

Yu Q., Shikuma C., Shiramizu B., and Hu N. Virolysis of complement-resistant HIV-1 by antibodies in the plasmas from HIV-1-infected individuals. 96<sup>th</sup> Annual Meeting of the American Association of Immunologists. May 8-12, 2009, Seattle, Washington. AAI Junior Faculty Travel Award from the American Association of Immunologists

Yu Q., Shikuma C., Shiramizu B., Yewdell J., Moss B., Hu N. A Potentially Negative Impact of Antiretroviral Therapy on Efficacy of Live Poxvirus-based Vaccines, Experimental Biology 2008, April 5-9, San Diego, CA, 2008.

## CONCLUSIONS

Task 1. Vietnam's National Institute of Hygiene and Epidemiology (NIHE) and the Hanoi School of Public Health (HSPH) are valuable research collaborators and together with UH significant research capacity has been developed in both places to identify febrile illnesses of unknown etiology. These research innovations include improved field surveillance and advanced sample identification at reference labs, and a novel and powerful microsphere-based immunoassay for improved dengue diagnostics.

Task 2. We have confirmed the importance of DENV virus genotype and its adaptive response in evolutionary time as a factor in DENV emergence. We have also revealed through advanced bioinformatics techniques that DENV experiences severe genetic bottlenecks that serve to reshuffle the deck, so to speak, of variation upon which selection acts, results in different evolutionary trajectories from population to population. Confirmation of the genetic basis of disease emergence and the role that adaptation of the pathogen may play has significant repercussions with respect to actionable decisions in the control of infectious diseases. With respect to dengue, alternative hypotheses for its emergence dictate different responses, from vaccination to mosquito control. Partnered with the power of genomics to detect adaptive strains and the parameters they are responding to (e.g., the genes that are evolving), control efforts could be empowered with greater effectiveness, targeting specific rapidly evolving populations. Adaptive virus strains are undergoing further phenotyping.

Task 3.1. We have generated recombinant ALVAC viruses expressing mouse CD40L and HIV-1 gag antigen, respectively. Augmented cytotoxic lymphocyte responses and CD4<sup>+</sup> T cell responses against HIV-1 in mice, and reaction in human monocyte-derived dendritic cells suggest that CD40L incorporation into ALVAC vectors could be used as a strategy to enhance HIV-1 vaccine immunogenicity.

Task 3.2. This pilot study demonstrated that endothelial dysfunction is common in HIV infected patients. The short-term use of extended release niacin can significantly improve endothelial function as measured by FMD in virologically stable HIV infected patients.

Task 3.1. We showed that high-throughput screening for HIV-1 in historical samples is both

effective and informative.

## **Appendices:**

Task 1:

Appendix I: Institutional Review Board (IRB) exemption from the U.S. Army, the University of Hawaii and from NIHE.

Appendix II: Detailed protocol including type of clinical samples to be taken, processing, transportation to NIHE in Hanoi, record keeping system, laboratory testing, case investigation forms, time lines for initiating phase II, inclusion and exclusion criteria, data storage, information technology and exchange, intellectual property rights issues and informed consent/forms.

Appendix III: Biosafety authorizations from the UH Institutional Biosafety Committee.

Appendix IV: Protocol translated to Vietnamese.

Appendix V: Agreements between the UH and NIHE through August 15, 2009.

Appendix VI: Trip Reports to Vietnam.

Task 2:

Appendix VII: Microsphere Immunofluorescent Assay (MIA) results.



Appendix I: Institutional Review Board (IRB) exemption from the U.S. Army, the University of Hawaii and from NIHE.

## Duchesneau, Caryn L Ms USAMRMC

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**From:** Duchesneau, Caryn L Ms USAMRMC  
**Sent:** Thursday, September 06, 2007 10:39 AM  
**To:** 'sbennett@hawaii.edu'  
**Cc:** Pascal, Louise M Ms AMDEX; Duchesneau, Caryn L Ms USAMRMC; Brosch, Laura R COL USAMRMC; Bennett, Jodi H Ms USAMRMC; Guertin, Kathy Ms USAMRAA; 'stanley.saiki@amedd.army.mil'; 'stephenson@tatrc.org'; 'yaaayin@hawaii.edu'; 'dgubler@hawaii.edu'; Wilberding, Julie A Dr AMDEX; Salai, Silvija Ms AMDEX; Smith, Catherine A Ms USAMRMC  
**Subject:** A-14273.1 HRPO Determination Memorandum Exempt (Proposal Log Number 06187003, Award Number W81XWH-07-2-0073) (UNCLASSIFIED)

Classification: UNCLASSIFIED  
Caveats: NONE

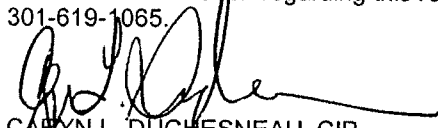
**SUBJECT:** Determination for the Protocol, "Advanced Technologies Addressing Asia-Pacific Infectious Diseases, Task 2 – Emerging Infectious Diseases: Dengue Virus (DENV) Genetic Study," Submitted by Shannon N. Bennett, PhD, University of Hawaii, Hawaii, in Support of the Research Proposal "Advanced Technologies Addressing Asia-Pacific Infectious Diseases," Submitted by Duane J. Gubler, ScD, Proposal Log Number 06187003, Award Number W81XWH-07-2-0073, HRPO Log Number A-14273.1

1. The subject protocol and supporting documents received on 21 August 2007 in the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO) have been reviewed for applicability of human subjects protection regulations.
  2. According to the protocol (version date 5 July 2007, page 5 and 6) this study will sequence isolates that have been archived in well-documented collections at the Asia-Pacific Institute of Tropical Medicine and Infectious Diseases (APITMID), and the Center for Disease Control (CDC) San Juan Branch in Puerto Rico and code them numerically with a sequential number. These anonymous archived isolates that were stripped of all individual and/or personal data will be selected randomly. They will be further stratified by year, endemic/epidemic phase of the populations and location. The study will use whole genome sequence 15 DENV isolates per year per endemic/epidemic cycle for each of up to four focal populations. The focal populations will vary both spatially and temporarily from Tahiti across the late 1970s to Puerto Rico between 1994 and 1998. The research team will use self designed long-read RT-PCR with primers for data collection according to standardized techniques (the design has been tested in previous studies and published – protocol page 6, first paragraph). By collecting 15 isolates per year, the researchers state that there is an 80% chance of sampling rare genotypes (defined as 10% of the population) at least once according to the binomial distribution. The data analysis is described in the protocol on page 6 under subheader 8.0 Data Analysis.
  3. In accordance with 32 CFR 219.101(b)(4), the HRPO determined that the protocol is exempt as it is research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.
  4. The HRPO concurs with the determination made by the University of Hawaii Institutional Review Board. The project may proceed with no further requirement for review by the HRPO. The HRPO protocol file for this specific project will be closed. If additional projects under this award involve non-exempt research, HRPO protocol files for these projects will remain open.
  5. In the event that there is a change to the subject research or statement of work (SOW), the Principal Investigator must notify the Contracting Officer's Representative (COR)/Grant Officer's Representative (GOR) and send a description of the change to the HRPO at [hsrrb@us.army.mil](mailto:hsrrb@us.army.mil) referencing both the Proposal Log Number and the HRPO Log Number listed in the "Subject" line above. The HRPO will re-open the protocol file if necessary.
- Any changes to the SOW that the COR/GOR determines could affect the exemption status of the project must be reviewed by the HRPO prior to approval by the Contracting Officer/Grants Officer.
6. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer/Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

7. Further information regarding the grant can be obtained by contacting the assigned Contract Specialist, Kathy Guertin, at 301-619-7434.

8. Further information regarding technical oversight can be obtained by contacting the assigned COR Stanley Saiki, Jr., MD, at 808-433-3600.

9. Further information regarding this review may be obtained by contacting Silvija Salai, RN, MBA, CCRC, at 301-619-1065.



CARYN L. DUCHESNEAU, CIP  
Chief, Human Subjects Protection Review  
Human Research Protection Office  
Office of Research Protections  
U.S. Army Medical Research and Materiel Command

Note: The official signed copy of this approval is housed with the protocol file at the Office of Research Protections, 504 Scott Street, Fort Detrick, MD 21702. Signed copies will be provided upon request.

Classification: UNCLASSIFIED  
Caveats: NONE

**Committee for the Protection of Human Subjects - University of Hawaii  
Declaration of Exemption**

Please go to Step 1, page 2, to confirm that research involves human subjects before completing form.

**GENERAL INFORMATION**

Name(s) of researcher(s): Duane J. Gubler, Sc.D., Director, Asia-Pacific Institute of Tropical Medicine and Infectious Diseases

Status: ☒ Faculty

Department: Tropical Medicine, Medical Microbiology, and Pharmacology

Mailing address: Asia-Pacific Institute of Tropical Medicine and Infectious Diseases,  
Dept. of Tropical Medicine, Medical Microbiology, and Pharmacology  
J.A. Burns School of Medicine  
University of Hawaii  
651 Ilalo St., BSB 320-G  
Honolulu, HI 96813

Phone number: 808-692-1674

Proposed Start Date: 16 July 2007

Email address: [dgubler@hawaii.edu](mailto:dgubler@hawaii.edu)

Title of research project: Advanced Technologies Addressing Asia-Pacific Infectious Diseases, Task 1 – Emerging Infectious Diseases: Develop a Pilot, Collaborative Zoonotic Disease Detection Program in the Asia-Pacific Region

**Application Review**

Application Approved ☐ Disapproved ☐

Exempt Category CFR 46.101(b) Yes ☐ No ☐

**Committee Comments/Recommendations:**

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CHS Committee Member \_\_\_\_\_ Date: \_\_\_\_\_  
(Signature)

CHS Staff \_\_\_\_\_ Date: \_\_\_\_\_  
(Signature)

Phone: (808) 956- 5007 e-mail: [dendle@hawaii.edu](mailto:dendle@hawaii.edu)  
Spaulding Hall, Room 253

## **Exempt Research**

The Committee for the Protection of Human Subjects (CHS) provides this form to investigators who plan to conduct "exempt" research involving human subjects. Use this form to determine whether your intended research involves human subjects and is exempt, and also to make a formal declaration of its status. This declaration helps both CHS and investigators in the task of separating research involving human subjects into two categories: 1) exempt research, which is not subject to regulation but which must be reported to the CHS nevertheless, and 2) non-exempt research, which must be submitted for full review and approval by the Committee before it begins.

Please follow all instructions carefully in making your declaration of exemption. This declaration will be checked for completeness and consistency, and incomplete or inconsistent forms will be returned to investigators.

**IMPORTANT NOTICE:** It is CHS and University policy that all research involving human subjects, whether exempt or non-exempt, funded or not, must be reported to the Committee. This rule has been instituted to protect both research subjects and investigators. Exempt research is not subject to regulation by the Committee (although it must still be reported). Non-exempt research, on the other hand, must be reviewed and approved by the CHS before it begins. Failure to report and receive Committee approval prior to the conduct of non-exempt research involving human subjects is a violation of University policy and, in some cases, federal law. By filling out this form and submitting it to CHS for verification prior to beginning your exempt research, you protect your own best interests as an investigator.

### **Step 1. CONFIRM THAT THE RESEARCH INVOLVES HUMAN SUBJECTS**

Does the research in question involve a living individual about whom an investigator (whether professional or student) conducting research activities obtains either:

- (1) Data through intervention or interaction with the individual (Intervention includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or environment that are performed for research purposes. Interaction includes communication or interpersonal contact between investigator and subject. ) or
- (2) Identifiable, private information? (Identifiable in this context implies that the identity of the subject is or may readily be ascertained by the investigator or associated with the information obtained as part of the research. Private information includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record).

**YES** ☒ **NO** ☐

If 'YES', you must either: (1) complete and submit this form if you believe that the research is exempt, or (2) submit a full protocol for review and approval by the CHS (please consult the Committee's Guidelines).

If 'NO', then the research does not involve human subjects, and there is no need to complete this form or to notify the CHS about the research, except in research that will involve the use of human blood, tissue or other pathological specimen. Such research requires CHS review and approval but may qualify for exemption if anonymous and pre-existing (see exemption category #6 for this, if applicable).

**NOTE:** It is possible to conduct research that involves living human beings who are not considered human subjects by definition. Examples of research on living human beings that may involve no interaction, intervention, or identifiable private information include: analyses of aggregate human data (e. g. census data, labor statistics), studies of public statements or declarations, analyses of individual-level data (even on private topics) if the respondents are not identifiable (e. g. , survey data distributed for public use). If you are unsure whether a research project involves human subjects or is exempt from regulation, you should call the CHS office at (808) 956-5007, or send an inquiry by email to [dendle@hawaii.edu](mailto:dendle@hawaii.edu).

In order to determine whether your research involving human subjects is exempt and to fulfill your responsibility to report exempt research to the CHS, please complete Steps 2 through 4.

Do any of the targeted populations for this research project consist of persons who are: legally incompetent; significantly mentally ill or impaired; or vulnerable to extraordinary institutional coercion, such as prisoners, residents of 24-hour skilled nursing facilities, or anyone who is involuntarily confined?

**YES** ☐ **NO** ☒

If 'NO', please continue.

## Step 2. CATEGORIES OF EXEMPTION

Please review the exemption criteria described in the CHS Guidelines before making a judgment that your project is exempt from full Committee review. These Guidelines, which are based on rules established by the federal government (see 45 CFR 46), specify that an investigation may be exempt from regulation if the only involvement of human subjects will be in one or more of the following categories:

1. Educational Practices
2. Educational Tests
3. Surveying or Interviewing
4. Public Observations
5. Public Officials
6. Existing Data

Please list applicable category number(s) of exemption that characterize this research project: 6

NOTE: All procedures for all subjects in this research project must be exempt in order to qualify for exemption. Further explanation of these exempt categories follows. Please proceed to Step 3.

## Step 3. JUSTIFICATION OF EXEMPTION

Fill out only those portions in Step 3 pertaining to the categories of exemption claimed in Step 2.

### 1. Educational Practices (1)

- A. Will this research involve the examination of school records for identifiable students, or the interviewing of instructors or other persons about identifiable students?  
YES ☐ NO ☐

If 'YES', then the project is not exempt and you must submit a complete protocol for review by the CHS.

If 'NO', then proceed to part B.

- B. Will the research be conducted in established or commonly accepted educational settings and involve normal educational practices, such as:

(i) Research on regular and special educational strategies; or

(ii) Research on the effectiveness of, or the comparison among, instructional techniques, curricula, or classroom management methods?

YES ☐ NO ☐

If 'YES', then the research is exempt and proceed to part C.

If 'NO', then the project is not exempt and you must submit a complete protocol for review by the CHS.

- C. Please provide adequate details, on an attached sheet, about the educational setting and practices involved in this research to justify your answer in part B above.

### 2. Educational Tests (2)

- A. Does the research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement) possess at least one of the following two conditions?

(i) In the researcher's private data (including field notes) as well as in any published material, information taken from these sources is recorded in such a manner that subjects cannot be identified, either directly or through identifiers linked to subjects; or

(ii) The information, if disclosed outside the research, could not reasonably place the subject at risk of criminal or civil liability or be damaging to the subject's financial standing, employability, or reputation.

YES ☐ NO ☐

If 'YES', then the research is exempt. Please indicate which condition(s) above apply \_\_\_\_\_ and proceed to part B.

If 'NO', then the project is not exempt and you must submit a complete protocol for review by the CHS.

- B. Please provide adequate details, on an attached sheet, about the educational testing involved in this research to justify answer in part A above.

### 3. Surveying or Interviewing (2) (3)

- A. Will any of the subjects involved in this research be minor children? If children will be excluded from this research through an initial screening mechanism, such as asking the age of respondents at the beginning of an interview, then the appropriate answer to this question is 'NO'. "Minor Children" are persons who have not attained the legal age for consent under the applicable jurisdiction in which the research will be conducted. In the United States, this age is 18 years.

YES ☐ NO ☐

If 'YES', then the project is not exempt and you must submit a complete protocol for review by the CHS.

If 'NO', please proceed to part B.

- B. Does the research involving surveying and interviewing possess at least one of the following two conditions?

- (i) In the researcher's private data (including field notes) as well as in any published material, responses are recorded in such a manner that subjects cannot be identified, either directly or through identifiers linked to subjects; or
- (ii) The responses, even if disclosed outside the research, could not reasonably place the subject at risk of criminal or civil liability or be damaging to the subject's financial standing, employability, or reputation.
- YES ☐ NO ☐

Note: Except in unusual political circumstances, surveys or interviews concerning attitudes on public issues are within section B. (ii) of this exemption.

If 'YES', then the research is exempt. Please indicate which condition(s) above apply \_\_\_\_\_ and proceed to part C.

If 'NO', then the project is not exempt and you must submit a complete protocol for review by the CHS.

- C. Please provide adequate details, on an attached sheet, about the surveying and interviewing involved in this research to justify your answer in part B above.

### 4. Public Observations (2)

- A. Will you participate in activities involving minor children at any time during the course of this research? If children will be excluded from this research through an initial screening mechanism, such as asking the age of respondents at the beginning of an interview, then the appropriate answer to this question is 'NO'. "Minor Children" are persons who have not attained the legal age for consent under the applicable jurisdiction in which the research will be conducted. In the United States, this age is 18 years.
- YES ☐ NO ☐

If 'YES', then the project is not exempt and you must submit a complete protocol for review by the CHS.

NOTE: Research involving observation of minors may qualify for exemption (if one of the conditions in part B. below is met). Any research involving participation in activities involving minors, however, is ineligible for exemption, except as provided under CHS exemptions #1 or #2.

If 'NO', please proceed to part B.

- B. Does the research involving the observation of public behavior possess at least one of the following two conditions?

- (i) In the researcher's private data (including field notes) as well as in any published material,

observations are recorded in such a manner that individual human subjects cannot be identified, either directly or through identifiers linked to the subjects; or

- (ii) The observations, even if disclosed outside the research, could not reasonably place the subject at risk of criminal or civil liability or be damaging to the subject's financial standing, employability, or reputation.  
YES ☐ NO ☐

If 'YES', then the research is exempt. Please indicate which condition(s) above apply \_\_\_\_\_ and proceed to part C.

If 'NO', then the project is not exempt and you must submit a complete protocol for review by the CHS.

- C. Please provide adequate details, on an attached sheet, about the public observations involved in this research to justify your answer in part B above.

#### 5. Public Officials (3)

- A. Are all of subjects of the research either elected or appointed public officials or candidates for public office? Please note that the staff of public officials, public administrators, and most personnel in state or federal agencies are not considered by the CHS to fit the definition of a "public official". If such persons will be subjects of research (perhaps in addition to public officials), then their participation may qualify separately for exemption under categories #2, #3, or #4, provided that this component of the research meets the appropriate criteria. Please complete all applicable pages of this form for the various human subjects involved in this project.  
YES ☐ NO ☐

Does the research involve educational tests, survey or interview procedures, or public observations?

YES ☐ NO ☐

If all answers above are 'YES', then the research is exempt and proceed to part B.

If all answers above are 'NO', the project does not qualify for this exemption and you must submit a complete protocol for review by the CHS, or seek another exemption category that may apply to this research.

- B. Please provide adequate details, on an attached sheet, about the research involving public officials in this research to justify your answers in part A above.

#### 6. Existing Data (4)

- A. Does the research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens possess at least one of the following two conditions?

(i) These sources (data, records, specimens, etc.) are publicly available; or

(ii) Both in the researcher's private data (including field notes) and in any published material, the information is recorded by the researcher in such a manner that subjects cannot be identified, either directly or through identifiers linked to the subjects.

YES ☒ NO ☐

If 'YES', which condition(s) above apply? ii Please proceed to part B.

- B. Please provide adequate details, on an attached sheet, about the use of existing data involved in this research to justify your answer in part A above.

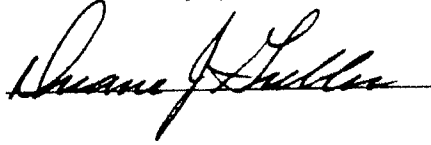
**Please attach a brief description or abstract of your proposed research. It must provide sufficient detail of the intent of the research and the involvement of human subjects to allow reviewers to make a thorough evaluation. If applicable, attach a copy of the survey instrument, consent and assent forms.**

**Send or deliver a signed original and 1 copy. Faxed/E-mail applications are not accepted.**



*I affirm that the above, to the best of my knowledge, is a true and accurate description of my proposed research.*

Signature of PI:



Date:

30/11/07

Have you completed:

UH Researcher training (4 hours)

YES ☐ NO ☒

NIH On-line training

YES ☒ NO ☐

### **Brief description of proposed research:**

In the past 15 years, there have been several regional or global epidemics that have for the first time, affected the global economy. Each of these epidemics, (plague in India, 1994, Avian influenza in Hong Kong, 1997, Nipah encephalitis in Malaysia, 1999, SARS IN China, 2002-2003, and Avian influenza, Southeast Asia, 2003-2007), have been caused by a zoonotic pathogen originating in Asia, and have had a progressively larger impact on the global economy. Our hypothesis is that there will be more of these regional/global epidemics because of globalization. Moreover, because of demographic, societal, economic and cultural factors, they will likely originate in Asia and be caused by zoonotic pathogens. By developing an effective early warning disease detection program in Asia, both known pathogens that cause human disease and currently unknown zoonotic pathogens that may have epidemic potential can be identified and controlled before major epidemic spread begins.

**Specific Aim:** Develop a pilot, collaborative zoonotic disease detection program in the Asia-Pacific Region.

### **Hypotheses:**

1. Zoonotic viruses with epidemic potential can be identified and controlled before major epidemic spread begins.
2. A pilot, zoonotic disease detection program can be established between APITMID and collaborating institutions in Viet Nam that will identify both previously recognized and new pathogens with epidemic potential.

### **Objectives**

1. Develop an active early warning syndromic disease detection system to monitor the infectious pathogens causing disease in humans in both urban and rural environments of northern Viet Nam
2. Use the latest laboratory technology to identify the pathogens causing febrile illness in humans.
3. Develop an information technology system that will allow real time communication and data exchange among the collaborating institutions.

In the country of interest, appropriate clinical samples will be collected in a sentinel surveillance system from patients who visit selected hospitals, clinics or private physicians for laboratory diagnosis using routine available laboratory diagnostic tests. Clinical samples from patients whose illness has an unknown etiology will be sent to APITMID in Honolulu for pathogen discovery with diagnostic testing that uses the latest technology, including immunohistochemistry, microarray, polymerase chain reaction (PCR), serology and isolation.

Clinical samples will be taken from patients seeking medical care at the sentinel sites for fever associated illness with emphasis on the following types of illness:

1. Viral syndrome
2. Acute respiratory illness
3. Dengue fever-like illness
4. Fever with or followed by hemorrhagic diathesis
5. Fever with or followed by neurologic disease
6. Viral prodrome followed by a fatal outcome

This surveillance effort does not select for any particular patients but will use samples from patients who are going through routine procedures used at their clinic to provide clinical samples for analysis.



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Hanoi, October 29<sup>th</sup>, 2007

**Institutional Review Board**  
**National Institute of Hygiene and Epidemiology**  
**Hanoi, Vietnam**

**Research proposal:** "Advance technologies addressing Asia-Pacific emerging infectious diseases"

*Funding source:* APITMID, Hawaii, USA  
*PI of Contract/Grant:* D.J.GUBLER

*Principal Investigator of Project:* Ass. Prof. NGUYEN TRAN HIEN, MD., PhD  
National Institute of Hygiene and Epidemiology,  
1 Yersin, Hanoi, Vietnam

The Institutional Review Board of National Institute of Hygiene and Epidemiology has reviewed and approved Dr. Nguyen Tran Hien's IRB application for the above Project, which involves human subjects research. We consider this to be a minimal risk study. This project is approved for being possible to carrying out ~~from the approved date.~~



TL, VIỆN TRƯỞNG

**Prof. Hoang Thuy Long, M.D., Ph.D.**  
**IRB Chair, NIHE**  
**Hanoi, Vietnam**



TRƯỞNG PHÒNG HCQT  
*Nguyễn Mạnh Cường*

Appendix II: Detailed protocol including type of clinical samples to be taken, processing, transportation to NIHE in Hanoi, record keeping system, laboratory testing, case investigation forms, time lines for initiating phase II, inclusion and exclusion criteria, data storage, information technology and exchange, intellectual property rights issues and informed consent/forms.

# **Viet Nam Collaborative Research & Training Program Using Advanced Technologies to Detect Emerging Infectious Diseases of Southeast Asia**

## **1. Introduction**

### **Background and Significance:**

Infectious diseases have been the leading cause of morbidity and mortality throughout history. With the advent of antibiotics and other new drugs, vaccines, insecticides and a better understanding of public health, however, infectious diseases were effectively controlled in most parts of the world by the 1960's.<sup>(1)</sup> Unfortunately, complacency and a redirection of resources to other competing public health priorities in the 1970s and 1980s led to a deterioration of the infectious disease infrastructure. This combined with powerful global trends in the past 30 years, e.g.:

- Unprecedented migration to, and population growth in, the cities of the developing world
- Uncontrolled urbanization
- Environmental change (deforestation, land and agricultural practices)
- Changing animal husbandry practices
- Other demographic and societal changes
- Modern transportation
- Globalization and the rapid and massive movement of people, animals and commodities

have contributed to a dramatic global re-emergence of epidemic infectious diseases in the last two decades of the 20th century.<sup>(1,2)</sup> Many diseases that were effectively controlled in the 1960's (cholera, tuberculosis, typhoid, malaria, dengue/dengue hemorrhagic fever, West Nile fever, yellow fever, Japanese encephalitis, plague, etc.) have re-emerged, causing major epidemics and increased morbidity and mortality. In addition, and perhaps more alarming, many newly recognized diseases have emerged as major public health problems during this time. These include AIDS, Ebola, Marburg, Lassa, Bolivian, Argentine and Venezuelan hemorrhagic fevers, Nipah encephalitis, SARS, avian influenza and a number of other viral, bacterial, and protozoal water-, food- and vector-borne infectious diseases. Finally, dramatic advances in biotechnology combined with socio-political issues have greatly increased the risk of deliberate release of biological agents. As we enter the 21st century, infectious diseases remain the most important cause of morbidity and mortality in the world.<sup>(3,4,5,6,9)</sup>

The majority of infectious disease epidemics in the past remained localized in the areas where they occurred naturally. However, because of the global trends mentioned above, the world has experienced several global public health emergencies in the past 25 years. The etiologic agents could have been contained at their point of origin had adequate laboratory-based disease detection and response systems been in place. These include in chronological order:

- The HIV/AIDS pandemic, 1980's – present
- The pneumonic plague epidemic, India, 1994
- The H5N1 avian influenza epidemic, Hong Kong, 1997
- The Nipah encephalitis epidemic, Malaysia, 1998-1999
- The SARS epidemic, China, 2002-2003
- The H5N1 avian influenza panzootic, 2003 – present

Only the 1997 Hong Kong avian influenza epidemic was contained before it spread. Of interest is that all of these epidemics were caused by zoonotic disease agents (pathogens of animals that jumped species to humans). More important is that with the exception of HIV/AIDS, all of these global public health emergencies have occurred in the past 15 years, have originated in Asia, and all have had an increasingly negative impact on the global economy.<sup>(1,2,4,7,8,9)</sup>

It is anticipated that the majority of infectious disease epidemics that have the potential to spread globally in the next 20 years will also be zoonoses that originate in Asia.<sup>(2)</sup> The rationale for this is that most of the world's population growth has occurred in the cities of Asia in the past 25 years, and the UN projects this trend will continue for the next 20 years. Of 57 megacities with populations of 5 million or more in 2000, 30 were in Asia. Many of these cities are populated by new immigrants from rural areas who bring their rural life style with them to the city, increasing the probability that exotic zoonotic disease agents will infect humans in a setting where epidemic transmission is facilitated by crowding, inadequate housing, water, sewer and waste management systems. All of these cities have modern international airports, and the dramatic economic growth in many Asian countries, combined with globalization and increased trade, increase the probability of infectious diseases spreading from Asia.<sup>(2)</sup>

## **2. Goal**

The goal of this Collaborative Research Program is to develop centers of excellence for research and training on emerging infectious diseases of the Asia Pacific region in Viet Nam, Hawaii and Singapore, and to use these centers to develop early warning infectious disease detection systems as well as to drive basic, field, and translational research.

### **Hypothesis:**

Pathogens with epidemic potential can be detected, identified and controlled before major epidemic spread begins by using new advanced diagnostic technologies.

## **3. Specific Aims**

### **Specific Aims:**

- a) Develop an active, early warning syndromic disease detection system to monitor the infectious pathogens causing disease in humans in both urban and rural environments of northern Viet Nam, Hawaii, and Singapore.
- b) Investigate the ecology/epidemiology of pathogens in Viet Nam that are considered to have epidemic potential, identifying natural hosts, and risk factors for transmission to facilitate development of prevention and treatment modalities
- c) Use a combination of classical and novel diagnostic methods to identify pathogens causing acute febrile illness in humans.
- d) Develop an information technology system that will allow real time communication and data exchange among the collaborating institutions and global surveillance programs.
- e) Develop programs to build laboratory and epidemiologic capacity, including technology transfer and bidirectional training programs that will serve the needs of Viet Nam, Singapore, and Hawaii

#### **4. Preliminary Results**

Active, laboratory-based disease detection systems that can provide early warning for epidemics of exotic diseases are desperately needed in most countries of Southeast Asia. The recent Nipah encephalitis, SARS, and avian influenza epidemics underscore the region's public health and economic vulnerability to the emergence of new pathogens. While we have no preliminary research results, it is well known that the majority of infections in Viet Nam and other countries in the region are of unknown etiology. The emergence of Nipah encephalitis and SARS demonstrate that some of these pathogens have significant epidemic potential. With appropriate early warning surveillance systems in place, these types of epidemics can be detected and contained before they spread too widely.

The approach will be to develop state of the art basic, field, and translational research capacity with a focus on zoonotic pathogens of Asia and the Pacific. At the heart of the program will be the field research center in Viet Nam which will develop the early warning disease detection system in Viet Nam, as well as conduct basic, field and translational research in settings where important infectious diseases occur naturally. Thus, this will form the foundation for a graduate training program that will train Vietnamese, Singaporean, and American students and scientists. This field center will be a rich source of research materials to drive pathogen discovery, diagnostic test, drug, and vaccine development for pathogens that have epidemic potential. This program will also be used to help build a strong biotechnology industry in the partner countries by facilitating partnerships among industry, academic and government institutions to translate basic research ideas from the bench to licensed products that can lessen the impact of emerging infectious diseases.

#### **5. Research Design and Methods**

##### **Study Design:**

This project will be carried out in three phases. Phase I will involve setting up the active disease detection systems. This phase has received IRB exemption status from NIHE, HSPH and the University of Hawaii, and can proceed without delay. Phase II will involve building laboratory and epidemiologic capacity, transferring the latest diagnostic and information technology, and conducting field epidemiologic and ecologic follow up investigations of pathogens that are considered to have epidemic potential. Phase II will only be implemented after phase I has been successfully implemented, and after full IRB approval has been obtained. Phase III will be a consolidation and maintenance phase, building on the progress achieved in Phase II to refine the early warning disease detection and response systems. Technology transfer will continue, and prevention and control strategies will be developed for those pathogens with epidemic potential. All three phases will involve training and capacity building in Viet Nam.

In Phase I, a syndromic surveillance system will be established to monitor febrile illness caused by infectious agents in North Viet Nam. Tentative sentinel surveillance sites include Bach Mai Hospital, Hanoi, Long Son Provincial Hospital, Viet Tiep City Hospital in Hai Phong, Hoa Binh District Hospital and Cao Bang District Hospital. In addition, selected health clinics, and private physicians will be used as sentinel sites in both urban and rural areas. Clinical samples will be taken from patients seeking medical care for fever associated illness at the sentinel sites. Emphasis will be given

to enrollment of patients with undifferentiated febrile illness occurring at an increased frequency suggestive of an outbreak and those with the following syndromes:

- a) Acute respiratory illness/pneumonia
- b) Fever with or followed by hemorrhagic diathesis
- c) Fever with or followed by neurologic disease
- d) Unexplained death or critical illness of probable infectious etiology

This system will require BSL-2 and BSL-3/ABSL-3 laboratories that can support the pathogen discovery program in Viet Nam, since many of the pathogens of public health and economic importance are classified by WHO and other international health agencies as level 3 pathogens. Moreover, many of the newly recognized pathogens that will be discovered in this program will likely be level-3 agents, and BSL-3 laboratories will be required for the safety of the technical staff.

Phases II and III will have full IRB approval. As such, the patient, clinical information and the sample will be linked to facilitate rapid and effective follow up. When a pathogen is considered to have important biological properties that may give it increased epidemic potential, a team will be assembled to conduct immediate field investigations. Depending on the pathogen and what is known about it, the field investigation team will be made up of scientists/physicians from Viet Nam, Singapore and Hawaii, and consist of epidemiologists, entomologists, laboratorians, behavioral scientists, vertebrate zoologists and others as needed. This team will conduct studies to determine the public health importance of the pathogen, including mode of transmission and identify possible intervention strategies for prevention and control. Every effort will be made to isolate the organism from natural hosts and to collect other materials that can be used to develop diagnostic assays, and if reasonable, develop new drugs and vaccines.

#### **Implementation Timeline:**

Phase I	2008
Phase II	2009 ( <i>upon completion of Phase I</i> )
Phase III	2010

#### **Study Methods:**

Depending on the laboratory capacity at NIHE and whether an epidemic is occurring, only a sample of the patients with undifferentiated febrile illness, acute respiratory illness and dengue-fever like illness will be taken. In general, 5 to 10 cases of each of these three syndrome types will be sampled per sentinel site each week. In addition, all patients with pneumonia, hemorrhagic manifestations, neurologic disease or with a fatal outcome associated with an infectious etiology who present to the sentinel sites will be sampled. This surveillance will continue throughout the entire year, unless there is an epidemic, at which time surveillance criteria will be modified according to the type of epidemic.

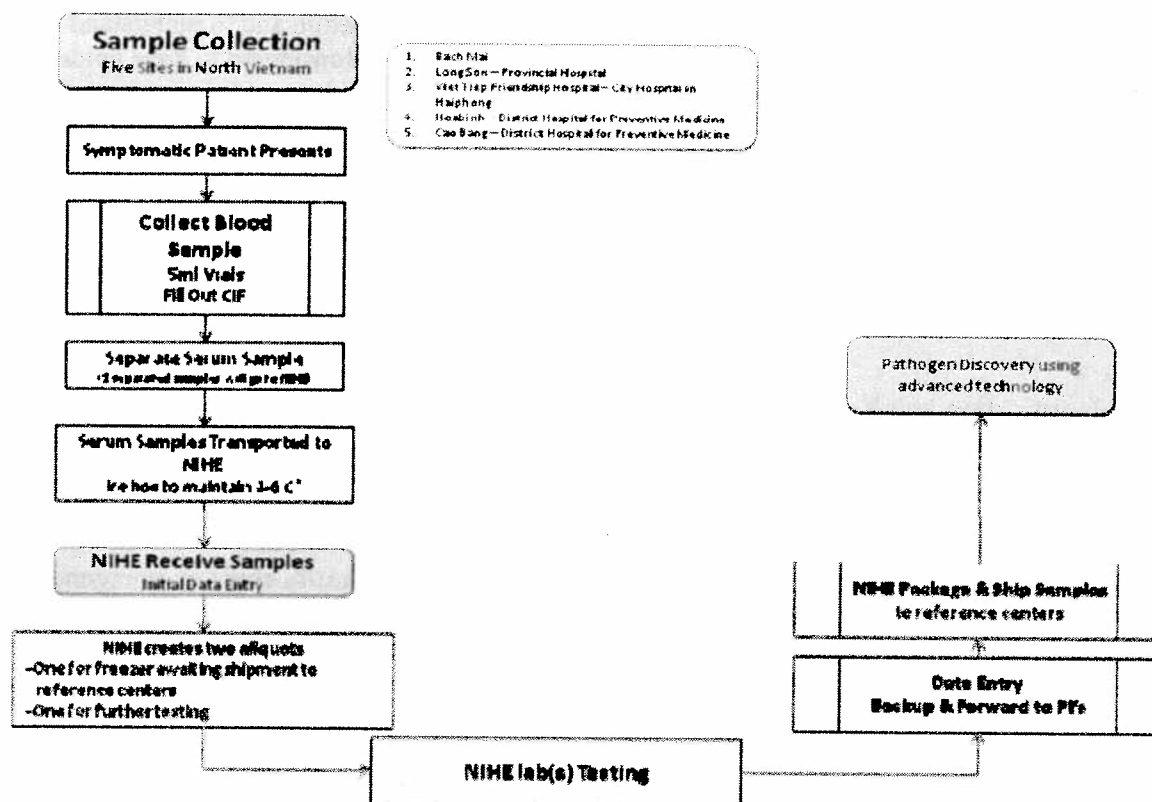
A Case Investigation Form (CIF) will be filled out on each patient, identifying which of the clinical syndromes noted above are suspected and briefly summarizing the clinical history, presentation and basic epidemiologic information. The CIF is attached as Appendix A. Clinical laboratory tests will be ordered as appropriate and the results recorded on the CIF by a nurse. Appropriate clinical samples (blood, throat washing/swab, cerebral spinal fluid (CSF) or tissue, depending on the syndrome) will be collected from patients, and stored in a refrigerator, freezer or a liquid nitrogen refrigerator,



depending on the site. Blood samples will be collected from all patients in 5.0 ml Red Top tubes, using a vacutainer, the sera separated from the clot and placed in a sterile tube for storage. All hospitalized patients will have blood samples drawn on admission and discharge to hospital. The CSF will be taken opportunistically, mostly from patients presenting with aseptic meningitis or encephalitis by lumbar puncture. Throat swabs and throat washings will be taken using commercially available collection materials. Tissues and heart blood will be taken opportunistically from patients who die, via autopsy or biopsy. Tissue samples will generally be stored in 10% formalin unless circumstances allow storage frozen at -70°C or in liquid nitrogen.

Briefly, all clinical samples will be processed as outlined in Figure 1. In Phases II and III, but not Phase I, the samples will be labeled with a number that allows them to be linked back to the patient and the home address for easy follow up. Using wet ice or ice packs, clinical samples will be shipped to NIHE on a weekly basis via courier where they will be divided into two aliquots; one aliquot will be stored for future studies at the reference laboratories; the other aliquot will be used for initial testing at various laboratories in NIHE for known diseases of public health importance, including, but not limited to dengue, West Nile, Japanese encephalitis, chikungunya, sindbis, hantaviruses, nipah virus, enteroviruses, influenza and other respiratory viruses, hepatitis B & C, typhoid, leptospirosis and rickettsia. Samples that require further study will be shipped to UH-APITMID or to Duke-NUS GMS, in accordance with international shipping regulations.

Figure 1. Algorithm for Collecting and Processing Clinical Samples at NIHE



The initial laboratory diagnostic testing will be conducted at NIHE using available laboratory diagnostic tests. In general, and depending on the suspected etiology, both serologic and virologic/bacteriologic/parasitological testing will be conducted. Serologic tests will include IgM and IgG ELISA, and neutralization tests. Virologic tests will include RT-PCR, virus isolation and immunohistochemistry as appropriate. Bacteriology and parasitological tests will be conducted based on clinical diagnosis. If the disease agent is identified as a known pathogen, e.g., dengue, influenza, Japanese encephalitis, malaria, leptospirosis, rickettsia, etc., follow-up epidemiologic and ecologic investigations will be initiated as appropriate by scientists from HSPH, NIHE, UH-APITMID, and Duke-NUS GMS after IRB clearance is obtained.

Clinical samples from patients whose illness has an unknown etiology will be sent to UH-APITMID or to Duke-NUS GMS in Singapore for pathogen discovery by Vietnamese, American and Singaporean scientists. Diagnostic testing using the latest technology, including genome and protein microarray analysis, Triangulation Identification for Genetic Evaluation of Risks (TIGER), immunohistochemistry, RT-PCR and isolation, will be conducted on each sample.<sup>(10,11,12)</sup> Unidentified agents will be sequenced and compared to other closely related microbial and viral agents. If they are new to science, they will be named and the work published in the peer reviewed journals with Vietnamese authors playing prominent roles.

In Phases II and III, newly recognized agents will be the focus of intensified surveillance and investigation in an attempt to isolate and characterize the agent in nature. Field investigation will be initiated by HSPH, NIHE, UH-APITMID and Duke-NUS GMS, after appropriate IRB approval, to identify natural hosts, mode of transmission, and importance to human health and to understand the basic ecology of the disease agent. Newly recognized microbial agents considered to have epidemic potential will also be the focus of basic research to develop immunogens that can be used in diagnostic tests for disease detection, or as potential candidate vaccines. Genome sequencing using advanced technology will be done at UH-APITMID or at the Genome Institute of Singapore by Vietnamese, American and Singapore scientists. Information will be shared with Vietnamese government agencies, as well as with U.S., Singapore and international health agencies such as WHO. Publication of results will be in peer reviewed journals with Vietnamese scientists playing prominent roles.

#### **Subject Identification and Recruitment:**

In Phases II and III IRB and consent will be obtained from patients or their representative prior to admission to the study. A draft consent form is presented in Appendix B.

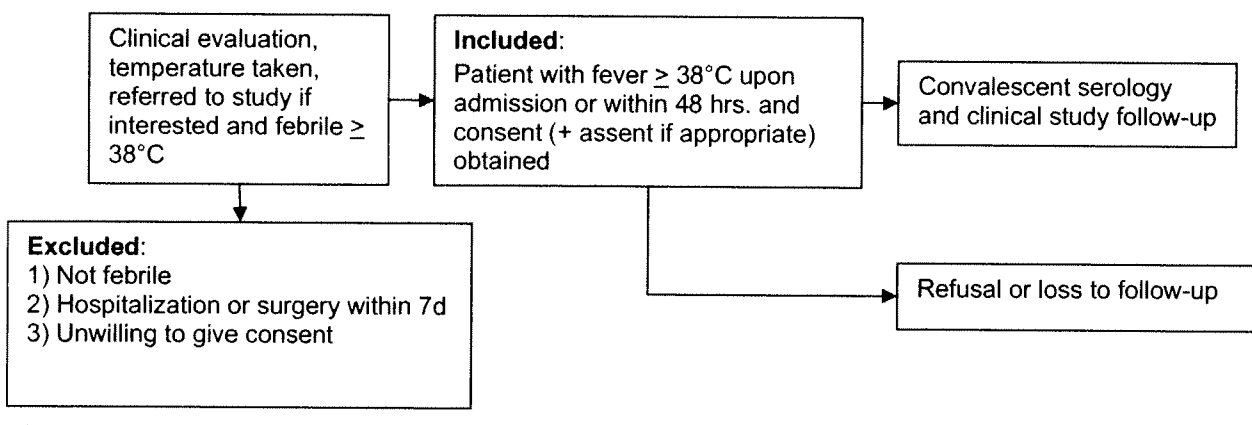
#### **Inclusion Criteria**

Patients  $\geq 2$  years of age presenting to hospital outpatient clinic, public health clinic or emergency will have their temperature measured and will be offered referral to study investigators following routine clinical evaluation if they are febrile (temperature  $\geq 38^{\circ}\text{C}$  tympanic or axillary, or  $\geq 38.5^{\circ}\text{C}$  oral). Patients admitted to the medical or the pediatric wards and found to have fever within 48 hours of admission will also be eligible and offered the opportunity to learn more about the study from study investigators. A history of febrile illness is not sufficient for inclusion. We aim to recruit 500-750 children  $\leq 14$  y.o. and 500-750 adolescents and adults  $>14$  y.o. during 2008. See Figure 2. Subject Identification and Recruitment.

### Exclusion Criteria

Patients currently hospitalized for >48 hours, patients who have been hospitalized or who have had surgery in the previous 7 days, and patients who are unable or unwilling to give consent for participation in both initial and convalescent evaluation.

Figure 2. Subject Identification and Recruitment



### Clinical Procedures, Treatment, and Monitoring:

#### Clinical Activities

Patients will receive routine clinical evaluation including physical exam and blood work from their routine care providers. Febrile patients will then be asked if they are interested in learning more about the fever study. Those interested will be referred to “pre-intern” study personnel (recently qualified physicians or nurses hired by, trained and supervised by senior study investigators). The study will be explained and participation offered.

#### Research Activities

A brief epidemiologic and clinical history and physical examination will be recorded on a standardized form after consent and assent is obtained. Study participants will then be referred to skilled care investigation phlebotomists (hired and trained by senior study investigators), who will obtain blood or other clinical samples for tests requested by the clinician and for study procedures in addition to sufficient blood for tests ordered by the clinician but not part of the study. Ten to 40 ml of venous blood will be drawn for complete blood count, biochemical tests (ALT, AST, Total bilirubin, creatinine), viral isolation, serology, PCR, peripheral blood smear, and aerobic blood culture [up to 10 ml (2 tsp) for 2-5 year olds, 15 ml (1 tablespoon) for > 5-10 year olds, 20 ml (1 1/3 tablespoons) for > 10-15 year olds, and 40 ml (2 2/3 tablespoons) for  $\geq 15$  year olds]. All patients will undergo diagnostic testing for numerous infectious pathogens but not for HIV. HIV testing will be limited to adults ( $\geq 18$  years) who give consent for HIV testing. Patients unwilling to give consent for HIV testing are still eligible for the study. Routine tests requested by the clinicians will not be duplicated.

Study participants will be requested to come for clinical and serologic follow-up at 2 weeks post onset of illness; telephone and written reminders will be used to assist in follow-up. Full convalescent serologies (repeat IgM and IgG) will be done only on patients whose initial febrile illness remains enigmatic on follow-up. Confirmatory serology will be done if locally-available and reported acute serology provided a presumptive diagnosis. Participants unable to return to hospital for follow-up will be visited at a more convenient location (e.g.-home) if possible. Results of all tests requested by the provider will be provided per routine; additionally, results of study blood culture and biochemical tests will be provided to patient's provider as soon as available (12-24 hrs, final blood culture result at 5 days). If the patient is found to be HIV positive the local STD campaign will be notified, will inform patients and take over care and counseling.

### **Risk/Benefit Assessment:**

#### **Risks:**

The risks of participating in this research study should be minimal and include the following:

- the insertion of the needle may cause temporary discomfort
- a bruise may form at the point where the needle enters the vein
- in rare cases, infection or fainting may occur

#### **Benefits:**

- Some, but not all, of the blood test results drawn for the purposes of the research study will be known by your doctor and may aid his/her decision of how to treat you.
- If you take part in this study, you may help others in the future who develop an illness with fever in Viet Nam by allowing health care professionals to better understand the causes of disease and the use of laboratory tests for disease with fever.

### **Ethical Considerations:**

#### **Subject Compensation**

Compensation in the order of current VND equivalent to USD \$3.00 will be provided to cover transport and time lost from work. All study procedures will be provided free of charge. Participants will be told that they may leave the study at any time.

#### **Subject Competency**

Parental consent will be sought for patients 2-18 years of age and additionally assent from those 12-18 years. Patients  $\geq 18$  years of age will give their own consent. See Appendix B: Draft Consent Form. Written consent or thumbprint (if illiterate) will be obtained. Pre-interns (recently graduated medical students) or nurses with experience will obtain consent and perform study procedures under direct supervision of our local site PI, who will also do initial training with them. Demonstrators (recently graduated medical students/ pre-interns) with pediatric experience will obtain consent and perform study procedures under direct supervision of our local site PI, who will also do initial training with them.

Ethical approval will be sought from NIHE, HSPH, UH, Duke University, and Duke-NUS GMS.

**Costs to the Subject**

There will be no costs to the patients for participation in this study. Patients will receive small compensation, as indicated above, to reimburse for any time lost from work and/or bus fare.

**Logistics and Training**

All sentinel sites used in the active surveillance system (Bach Mai Hospital, Hanoi, Lang Son Provincial Hospital, Viet Tiep City Hospital in Hai Phong, Hoa Binh District Hospital and Cao Bang District Hospital) will be provided with vacutainer tubes for blood collection, materials required for serum separation, storage refrigerators and/or freezers as necessary and shipping boxes. All consumable supplies will be disposable sterile plastic. Case investigation forms, labels and office supplies will be provided as necessary.

A nurse at each site will be trained to collect and process the clinical samples according to the protocol. That person will also be trained to collect required clinical and epidemiologic information on the patient to fill out the case investigation forms and to label the clinical samples according to protocol. Finally, this individual will be trained to explain the study to the patient or the patient's representative, and to obtain informed consent if appropriate.

Detailed protocols will be drafted for each of the procedures to be carried out. Field staff will be compensated according to Vietnamese pay scales determined by collaborators at NIHE.

**Data Analysis and Monitoring**

Data will be prospectively collected on the CIF (Appendix A). Data will be double entered into Epi Info™ on site in Hanoi, Viet Nam.

We will calculate the point prevalence and 95% confidence intervals for infectious causes of fever identified in this cohort. We will calculate odds ratios and 95% confidence intervals for associations between specific infections and patient characteristics, epidemiologic exposures, and physical symptoms and signs by exact methods and, when feasible, by multivariable logistic regression. We will compare the relative yield of diagnostic tests (paired sera vs. PCR by McNemar's chi-squared test).

**Data Storage and Confidentiality**

All patients will be identified by a unique study ID number. The only link to the patients' identifying information will be a face sheet that remains at the local study site; all other data, including blood samples and questionnaires will be identified only by the unique study ID number. These will be stored in a locked cabinet which will only be accessible to the study personnel. This information will be used to facilitate contacting patients with laboratory results and to assist clinical follow-up.

Some of each patient's blood sample will be tested locally at NIHE; these tests will be made available to the patient's doctors to improve their care.

The rest of each patient's blood sample will be sent to the reference laboratories (UH and Duke-NUS GMS) in the United States and Singapore, respectively, to conduct tests that are not currently available in Viet Nam (*see above*) to determine the etiology of febrile illness. Since these tests take time and the results are not yet fully understood, these results will not be available for patient care. These clinical samples will be retained by the study team until testing is complete or until they are

completely depleted, so that the best tests for rapid diagnosis of pathogens (organisms) causing fever in Viet Nam can continue to be evaluated. Patients are free to notify the principal investigator in writing if they would like to withdraw their data and blood samples from this research at any time. No human DNA testing will be performed.

A major goal of this collaboration is to upgrade laboratory and epidemiologic capacity in Viet Nam. Commensurate with this goal, the investigators intend to perform as many studies on site as possible with the intention of achieving a sustainable program. Routine bacterial and serological studies will account for the majority of the studies done on site. The sites are listed in order of preference above.

### **Information Technology and Exchange**

The latest information technology (IT) will be set up to link the reference centers with the Vietnamese sites. Data entry and analysis will thus be shared among the sites and participating scientists and physicians on a real time basis. Computer software will be developed to allow the computer to assign the patient ID numbers and the clinical sample accession numbers. In this manner, there will be real time exchange of clinical, epidemiologic, ecologic, diagnostic laboratory and analysis data among the participating scientists and physicians. As noted above, however, only the principal investigators in Viet Nam will have access to the confidential information that links the patients to an ID number.

## **6. Expected Results and Limitations**

The Viet Nam disease detection system will generate a wealth of research materials for basic and applied research as well as for developing new diagnostic tests, drugs, vaccines, etc. These materials will be shared with scientists in Viet Nam, Hawaii and Singapore. In addition, the field sites will provide platforms for clinical trials and other translational research as well as for training scientists and physicians in the laboratory and clinical diagnosis and management of a wide variety of infectious diseases that could be spread globally. Collectively, this group of partners provides a very strong infrastructure that will help insure success.

This study will provide data on the etiology, prevalence, and clinical characteristics of pediatric and adult patients with febrile illness in northern Viet Nam. The process will contribute to the improvement of diagnostic microbiology facilities and the development of national and regional guidelines for management of patients who present with fever. In addition, the data generated by this study will effectively delineate the prevalence of infections suspected to be frequent, based on serologic data, for which confirmatory studies have not previously been possible. The application of molecular amplification methods is expected to provide a better defined spectrum of pathogens that should contribute to the strategic implementation of evidence-based therapies and prevention.

The success of this program will place Viet Nam, Hawaii and Singapore in a political, economic, basic science research and public health leadership role in Southeast Asia. The successful program will generate “cutting edge” research in academic, government and private institutions, which using the latest technology, should produce a pipeline of new products, all of which will have to be tested in the field, and which will be co-owned by all partners. The successful program will also result in working partnerships with other countries in the region, which combined with the two-way training programs, will produce trusting and workable relationships in both the public and private sectors.

Finally, the successful program will produce early warning disease surveillance systems in the region that will allow detection and rapid response to emerging pathogens, thus preventing the spread of epidemic diseases such as SARS.

## **7. Closure**

This project is designed to develop a disease detection system in the partner countries that will provide early warning for epidemic transmission of exotic diseases before they begin to spread. It requires laboratory and epidemiologic capacity building, especially in Viet Nam. Assuming the successful implementation of the project, it is anticipated that the project will provide the materials that will drive a major research and training program that will result in development of new technologies, diagnostics, therapeutics, immunogens and vaccines.

## **8. Partners**

Important stakeholders that will add considerable strength to this program include:

1. The National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam; NIHE is the premier institute in Viet Nam for infectious disease surveillance, diagnosis, prevention and control. It has enjoyed laboratories, well trained scientists, a BSL-3 laboratory and is respected as the leading infectious disease institute in the country.
2. The Hanoi School of Public Health, Viet Nam; HCPH is the principal source of training in epidemiology, surveillance, prevention and control in Viet Nam. It has a growing cadre of young epidemiologists eager to participate in the ecologic and epidemiologic follow up studies.
3. Viet Nam Administration of Preventive Medicine, Hanoi; The Viet Nam administration of Preventive Medicine is responsible for all preventive medicine centers in the country and as such, is responsible for laboratory and epidemiologic capacity building in Viet Nam.
4. University of Hawaii at Manoa, USA; The University of Hawaii has long standing relationships with countries in Southeast Asia, providing training for many health professionals in the region. The university has strong cultural and economic ties in the region. It is also home to the Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, which focuses on infectious diseases of the Asia- Pacific region.
5. Duke - National University of Singapore (NUS) Graduate Medical School (GMS); The Duke-NUS Graduate Medical School is a new collaborative venture between Duke University in the USA and National University of Singapore to develop a new Graduate Medical School the will emphasize research and will focus on training physician scientists. It will have five signature research programs, including Emerging Infectious Diseases.
6. Duke University, Durham, North Carolina, USA; In addition to the Duke/NUS campus in Singapore, Duke University brings considerable resources and expertise to the program. The Duke Global Health Institute (DGHI) which serves as an umbrella entity for global health at Duke was

created in 2006 to address health disparities around the world. As a university-wide institute it brings faculty and students from all nine schools at Duke in the areas of research, education, service, and policy. The Hubert-Yeargan Center for Global Health (HYC) is an effecter arm for the medical center at Duke and provides bidirectional educational opportunities for medical students and graduate level trainees. The Duke Institute for Genome Science and Policy (IGSP) brings an applied science approach to support campus-wide scholarship exploring the impact of genome sciences on all aspects of life, human health, and social policy.

7. Singapore; The various institutions in Singapore that have infectious disease and technological expertise, including but not limited to the Communicable Disease Centre, the Genome Institute of Singapore, the National University Hospital, the NUS Department of Microbiology, the Novartis Institute of Tropical Diseases, the Environmental Health Institute, the DSO National Laboratories, the Singapore Immunology Network, the Singapore Institute for Clinical Sciences, SingHealth and others. All have technical expertise that will be critical to developing a research center of excellence in Singapore.

8. East-West Center Honolulu, Hawaii; The East – West Center Honolulu, Hawaii, – adds expanded dimension to ties with Asia

9. The Southeast Asian and the Western Pacific Regional Offices and Headquarters of WHO in New Delhi, Manila and Geneva; The Southeast Asian and the Western Pacific Regional Offices and Headquarters of WHO in New Delhi, Manila and Geneva, respectively, are needed to work closely with the Duke-NUS program on disease surveillance and control in the Asian-Pacific Region.

## **9. Personnel**

Prof Nguyen Tran Hien, Principal Investigator  
Prof Duane J Gubler, Co-Principal Investigator  
Prof Truong Uyen Ninh, Project Coordinator  
Prof Le Vu Anh, Co-Investigator  
Prof Vu Sinh Nam, Co-Investigator  
Prof Christopher Woods, Co-investigator

## **10. Intellectual Property and Discovery**

1. Scientific and technical data generated by this research and other scholarly activities are the currency of the intellectual capital that researchers and scholars create and share to advance the research enterprise. Both investigators from the University of Hawaii and the Vietnamese National Institute of Hygiene and Epidemiology (NIHE) have shared rights and responsibilities with respect to research data originating from the activities executed within this agreement.



- 1.1 The University of Hawaii and NIHE investigators equally share obligations regarding retention of and providing access to research data, samples, and information.
  - 1.2 Any news release, public announcement, advertisement or publicity released by either party concerning this Agreement, or any proposal under this Agreement, or any resulting contract or subcontract, will be subject to notification of the other party. Any such publicity shall give due credit to the contribution of each party.
  - 1.3 Rights to discoveries – Unless otherwise stipulated in writing and agreed to by the parties, any discoveries arising during the term of the Agreement shall be the joint property of NIHE, Hanoi School of Public Health (HSPH), University of Hawaii and Duke-National University of Singapore (NUS) Graduate Medical School; each shall have an equal interest in and to such discoveries.
  - 1.4 The parties agree to abide by provisions in the Prime Contract that may require the parties to grant license or other rights in the discoveries and associated data to the U.S. Government.
2. This agreement defines “data” as defined as the recorded factual material commonly accepted in the scientific community as necessary to validate research findings, but not any of the following: preliminary analyses, drafts of scientific papers, plans for future research, peer reviews, or communications with colleagues.”

## 11. References

1. *Microbial Threats to Health: Emergence, Detection and Response*. Institute of Medicine, The National Academies Press; 2003.
2. Gubler, DJ 2008a. The Global Threat of Emergent/Reemergent Vector-borne Diseases. pp 43-64. In: Institute of Medicine. 2008. *Vector-borne diseases: understanding the environmental, human health, and ecological connections*. Washington DC: The National Academies Press.
3. National Advisory Committee on SARS and Public Health (Canada). Learning from SARS – Renewal of Public Health in Canada – A report of the National Advisory Committee on SARS and Public Health. 2003. Ref. Type: Report.
4. *The Emergence of Zoonotic Diseases: Understanding the Impact on Animal and Human Health*. Institute of Medicine, National Academy Press; 2002.
5. Gubler, DJ. Global Emergency/Resurgency of arboviral disease as public health problems. *Arch Med Res*. 2002; 33:330-342.
6. WHO. 2002. The World Health Report 2002. World Health Organization, Geneva, Switzerland.
7. Guber, DJ 2008b. The 20<sup>th</sup> century re-emergence of erthropod-borne viral diseases: lessons learned and prospects for the future. In: *Proceedings of the Eight Sir Dorabji Tata Symposium on Arthropod-borne Viral Infections, Bangalore, India*, edited by D. Raghunath and C. Durga Rao, Sir Dorabji Tata Centre for Research in Tropical Diseases. Bangalore, India, McGraw-Hill publishing Co. Ltd.
8. Salaam-Blyther, T. and Chanlett-Avery E. U.S. and International Responses to the Global Spread of Avian Flu: Issues for Congress—CRS Report for Congress. 1-2-0006. Washington, D.C., Foreign Affairs, Defense, and Trade Division, Congressional Research Service, the Library of Congress. Ref Type: Report
9. NFID (National Foundation for Infectious Diseases). Current and future infectious disease threats: developments in the fight against infectious diseases. The 10<sup>th</sup> Richard J. Duma/NFID Annual Press Conference and Symposium on Infectious Diseases, Washington DC, July 27, 2005. 2005.
10. Jessie K, Fong M, et.al. Localization of dengue virus I naturally infected human tissues, by immunohistochemistry and in situ hybridization. *J Inf Dis*. 2004; 189:1411-1418.
11. Palacios G, Briese T, et.al. MassTag Polymerase Chain Reaction for Differential Diagnosis of Viral Hemorrhagic Fevers. *Emerging Infec Dis*. 2006;12:692-695.
12. Wang D, Coscoy L, et.al. Microarray-based detection and genotyping of viral pathogens. *PNAS*. 2002;99:15687-15692.

## CASE INVESTIGATION FORM

Study ID: _____	Date (DD/MM/YY) _____	Seen/Exam By: (initials) _____
Fever Study: _____	Hospital/Clinic/ Physician _____	Reviewed by: _____
		Data Entry: _____

## Confidential Face Sheet Identifying Information

1. Patient Name (Last) \_\_\_\_\_
2. Patient Name (First) \_\_\_\_\_
3. Hospital ID: \_\_\_\_\_ (OPD# or BHT#): \_\_\_\_\_
4. DOB: (DD/MM/YY) \_\_\_\_\_
5. Occupation: \_\_\_\_\_
6. Consent given by: ☐ Self (Patient  $\geq 18$  y.o.) \_\_\_\_\_  
☐ Other (caregiver  $> 18$  y.o.) \_\_\_\_\_  
If "Other": Name (Last, first) \_\_\_\_\_
7. Assent given by: (child  $\geq 8$  y.o. and developmentally normal) ☐ Yes ☐ No
8. Patient's home address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_
9. Patient/family telephone number: ☐ Patient's home telephone no.: \_\_\_\_\_  
☐ Other; specify relation/friend: \_\_\_\_\_ telephone no: \_\_\_\_\_  
☐ Work; specify \_\_\_\_\_ telephone no. \_\_\_\_\_  
☐ School; specify \_\_\_\_\_ telephone no. \_\_\_\_\_
10. HIV test offered: ☐ Yes ☐ No  
Pretest counseling done ☐ Yes  
Follow-up arranged ☐ Yes Specify: \_\_\_\_\_

## CASE INVESTIGATION FORM

Study ID: _____	Date (DD/MM/YY) _____	Seen/Exam By: (initials) _____
Fever Study: _____	Hospital/Clinic/ Physician _____	Reviewed by: _____
		Data Entry: _____

**Background/Epidemiology**

Inpatient/Outpatient: OPD = 1  
Admit = 2

Age: \_\_\_\_\_ yrs \_\_\_\_\_ mos

Sex: ☐ M ☐ F

Residence: ☐ urban ☐ rural  
☐ other

Work: ☐ home ☐ school  
☐ merchant  
☐ farming; type \_\_\_\_\_  
☐ laborer; type: \_\_\_\_\_  
☐ other; specify \_\_\_\_\_

Education: ☐ < O/L ☐ O/L  
☐ A/L ☐ > A/L

Exposure in/around home:  
☐ pig ☐ dog ☐ cat  
☐ goat ☐ cow ☐ rodent  
☐ other; specify \_\_\_\_\_

Exposure to fresh water:  
*swim/bathe/wade*  
☐ river ☐ paddy ☐ field  
*drink or consume*  
☐ tap ☐ well ☐ bottled ☐ boiled

**Symptoms in Last Month**

1° Symptom: \_\_\_\_\_  
onset date: (dd/mm/yy) \_\_\_\_\_

Review	Yes	#Days	No	Unsure
Fever/chills	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Sudden hearing loss	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Cough: Dry	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Productive	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Bloody	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Sore throat	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Short of breath	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Vomiting	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Diarrhea (≥3x/24hr)	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Abdominal pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Painful urination	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Decreased urination	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Headache	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Lethargy	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Convulsions	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Joint pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Muscle pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Rash	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Hemorrhagic manifestation	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

Antibiotic for this illness: ☐ Yes ☐ No ☐ Unsure

☐ amox ☐ sulfa ☐ 2<sup>nd</sup> /3<sup>rd</sup> Ceph  
☐ dox/tetracycline ☐ azithro/erythro  
☐ fluoroquinolone ☐ other

Name of antibiotic: \_\_\_\_\_

Last dose: \_\_\_\_\_ hours

**Examination**

Temperature: _____ °C <input type="checkbox"/> oral <input type="checkbox"/> tym <input type="checkbox"/> axillary Midarm circumference: _____ cm <i>If patient is &lt; 18</i> Resp rate: _____ HR BP _____ Ht _____ Wt _____ <i>metric ht/wt</i> Conjunctival injection/suffusion: <input type="checkbox"/> Yes <input type="checkbox"/> No Infection of the throat? <input type="checkbox"/> Yes <input type="checkbox"/> No Infection of the ear? <input type="checkbox"/> Yes <input type="checkbox"/> No Nodes: <input type="checkbox"/> none <input type="checkbox"/> cervical <input type="checkbox"/> diffuse <input type="checkbox"/> other	CHEST crackles: <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure ABDOMEN enlarged: <input type="checkbox"/> Yes <input type="checkbox"/> No _____ cm Liver enlarged: <input type="checkbox"/> Yes <input type="checkbox"/> No _____ cm Spleen enlarged: <input type="checkbox"/> Yes <input type="checkbox"/> No _____ cm Pain w/palpitation: <input type="checkbox"/> Yes <input type="checkbox"/> No CNS – neck stiffness <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure <input type="checkbox"/> alert <input type="checkbox"/> confused <input type="checkbox"/> drowsy <input type="checkbox"/> coma SKIN/JOINT: rash <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure <input type="checkbox"/> eschar <input type="checkbox"/> diffuse <input type="checkbox"/> petechial <input type="checkbox"/> Jaundice/Icterus <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure <input type="checkbox"/> Skin pustule/abscess <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure <input type="checkbox"/> Hot swollen joint <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unsure
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## CASE INVESTIGATION FORM

Study ID: _____	Date (DD/MM/YY) _____	Seen/Exam By: (initials) _____
Fever Study: _____	Hospital/Clinic/ Physician _____	Reviewed by: _____
		Data Entry: _____

**Clinical Diagnosis (before test results)**

<input type="checkbox"/> Viral fever	<input type="checkbox"/> Dengue
<input type="checkbox"/> Malaria	<input type="checkbox"/> Enteric fever
<input type="checkbox"/> Scrub typhus	<input type="checkbox"/> Leptospirosis
<input type="checkbox"/> Other _____	

**Laboratory Results:**

ND=Not Done

Malaria: <input type="checkbox"/> Pos	<input type="checkbox"/> Neg	<input type="checkbox"/> ND
HIV: <input type="checkbox"/> Pos	<input type="checkbox"/> Neg	<input type="checkbox"/> ND

**Follow-up (Convalescent) Visit**

Date: (dd/mm/yy): \_\_\_\_\_

☐ Normal Health ☐ Continuing Symp \_\_\_\_\_

Duration of Hospitalization: ☐ None Days \_\_\_\_\_

Still Symptoms	Yes	Total Days	No	Unsure
Fever/chills	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Sudden hearing loss	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Dry:	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Cough:	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Productive	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Bloody	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Sore throat	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Short of breath	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Vomiting	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Diarrhea ( $\geq 3x/24hr$ )	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Abdominal pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Painful urination	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Decreased urination	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Headache	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Lethargy	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Convulsions	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Joint pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Muscle pain	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Rash	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Hemorrhagic manifestation	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

Convalescent serum collected ☐ Yes \_\_\_\_ ml ☐ No

**Any change in signs/symptoms/New clinical findings?**

☐ Yes ☐ No \_\_\_\_\_

**Relevant Physical**

WBC \_\_\_\_ N \_\_\_\_ Band \_\_\_\_ L \_\_\_\_ E \_\_\_\_ M \_\_\_\_

Hb \_\_\_\_ Plts \_\_\_\_ ALT \_\_\_\_ AST \_\_\_\_ Tbili \_\_\_\_ Cr \_\_\_\_

Blood culture: ☐ Org(s) \_\_\_\_\_ ☐ Neg ☐ ND

Urine: Ag Pnemo ☐ Pos ☐ Neg; Legionella ☐ Pos ☐ Neg

Dip ☐ Protein ☐ Blood ☐ Other \_\_\_\_\_

Chest x-ray ☐ Done \_\_\_\_\_

**Therapy given** ☐ Yes ☐ No; #days \_\_\_\_\_

<input type="checkbox"/> Chloramphenicol	<input type="checkbox"/> Amox/Penicillin
<input type="checkbox"/> Erythromycin	<input type="checkbox"/> Ciprofloxacin
<input type="checkbox"/> Ceftriaxone	<input type="checkbox"/> Cefuroxime
<input type="checkbox"/> Gentamicin	<input type="checkbox"/> Doxy/Tetracycline
<input type="checkbox"/> TMP-SMX	<input type="checkbox"/> Other: _____

**Plan:** ☐ Home ☐ Refer ☐ Admit

If admit: dd/mm/yy \_\_\_\_\_

Discharge: dd/mm/yy \_\_\_\_\_

Planned Follow-up: \_\_\_\_\_  
(@2-4weeks)

**Findings:** \_\_\_\_\_

Any investigations ordered (from diagnosis card)? ☐ Yes ☐ No

If yes: \_\_\_\_\_

Therapy given: ☐ Yes ☐ No

If yes: \_\_\_\_\_

**PCR Results:** Date as: DD/MM/YY

Blood <input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> Neg
Blood <input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> Neg
Blood <input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> Neg
Blood <input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> Neg

**Serologic Results:** Date as: DD/MM/YY

<input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> IgM <input type="checkbox"/> IgG
<input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> IgM <input type="checkbox"/> IgG
<input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> IgM <input type="checkbox"/> IgG
<input type="checkbox"/> / <input type="checkbox"/> / <input type="checkbox"/>	Org: _____	<input type="checkbox"/> IgM <input type="checkbox"/> IgG

**Urine antibodies present:** ☐ Yes ☐ No

Chest x-ray: ☐ NI ☐ Abnl \_\_\_\_\_

**CSF results – only if performed by clinician**

CSF Glu \_\_\_\_ Pr \_\_\_\_ Gram st \_\_\_\_\_

WBC \_\_\_\_ N \_\_\_\_ L \_\_\_\_ M \_\_\_\_ RBC \_\_\_\_ ND \_\_\_\_

CSF culture ☐ Org \_\_\_\_\_ ☐ Neg

**Final Diagnosis**

<input type="checkbox"/> Unknown	<input type="checkbox"/> Dengue	<input type="checkbox"/> Malaria
<input type="checkbox"/> Enteric fever	<input type="checkbox"/> Scrub thy	<input type="checkbox"/> Leptospirosis
<input type="checkbox"/> Pneumonia	<input type="checkbox"/> Sepsis	<input type="checkbox"/> Meningoenceph
<input type="checkbox"/> Urine infect'n	<input type="checkbox"/> Other: _____	

## CASE INVESTIGATION FORM

Study ID: _____	Date (DD/MM/YY) _____	Seen/Exam By: (initials) _____
Fever Study: _____	Hospital/Clinic/ Physician _____	Reviewed by: _____
		Data Entry: _____

**Final Diagnosis**

- ☐ Unknown    ☐ Dengue    ☐ Malaria  
☐ Enteric fever    ☐ Scrub typhus    ☐ Leptospirosis  
☐ Pneumonia    ☐ Sepsis    ☐ Meningoencephalitis  
☐ Urine infection    ☐ Other: \_\_\_\_\_

Organisms(s): \_\_\_\_\_

**Patient outcome:**

At week # \_\_\_\_\_; final follow-up: \_\_\_\_\_

- 1= Died  
 2= Discharged  
 3= Inpatient  
 4= Absconded

**If hospitalized, bed head ticket review performed:**

☐ Yes    ☐ No

**Notes:**

e.g. other relevant features of initial history and physical examination, hospitalization, or clinical course not addressed above. (*selected cases only*)

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## Appendix B

# RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

**Protocol Title:** Using Advanced Technologies to Detect Emerging Infectious Diseases in Viet Nam  
**Application no.**  
**Sponsor:**  
**Principal Investigator:** Dr. Nguyen Tran Hien for NIHE, Dr. D.J. Gubler for UH  
**Date:**

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**Dear Study Participant,**

**What you should know about this study:**

- You are being asked to join this research study because either you or your child has a fever.
- Research studies include only people who agree to participate. This consent form explains the research and what you would need to do if you agree to participate. Please read it carefully and take as much time as you need. Ask questions at any time about anything you do not understand.
- You are a volunteer. If you join the study, you can change your mind later. You can decide not to take part or you can quit at any time. There will be no penalty or loss of benefits if you decide to quit the study.
- While you are in this study, the study team will tell you any new information that could affect whether you want to stay in the study. If your child can join this study, the word “you” in this consent form will refer to both you and your child.

**Why is this research being done?**

- Illnesses associated with fever cause a lot of sickness and occasionally death.
- Causes of fever differ by region, climate, and age. Some causes of fever require urgent treatment and medication, while others require no specific treatment at all.
- This research is being done to better understand the causes of illness with fever in Viet Nam.
- Knowing the major causes of fever in children and adults will help us learn how to best treat people and where to focus efforts to prevent and treat illness.

**How many people will take part in this study?**

- 500-750 patients of ages 2-14 years and 500-750 patients over age 14 years will be enrolled over a 3-4 month period in the dry season and the same number in the wet seasons of 2007/8.

**What will happen if you join this study?**

- If you agree to be in this study, you will be asked to sign this consent form. You will then receive your routine medical care from your doctor at THIS VISIT which may include:
  - collecting information about your medical history.
  - a physical examination.
  - blood tests

The routine part of your care for this visit will be provided free of charge by the hospital as usual. Your doctor will make decisions about how to treat you from the information collected during this visit.

**RESEARCH FEVER STUDY PROCEDURES:**

Medical information for the Research Fever Study will be obtained by a qualified physician by administering a questionnaire and performing a physical examination in addition to your routine medical care.

**If you agree to be in the Research Fever Study, we will need to collect some extra blood samples to help us better understand the causes of infections that cause fever. We will also ask you to come back for a SECOND visit in 2-4 weeks.**

**Research Procedures THIS VISIT:**

- Blood will be drawn by an experienced phlebotomist (a retired nurse). The total amount of extra blood taken from a vein in your arm for this research study will be about 10 to 40 ml ( $\frac{2}{3}$  to  $2\frac{1}{3}$  tablespoons), i.e. about 10 ml ( $\frac{2}{3}$  tablespoon) for 2-5 yo, 15 ml (1 tablespoon) for 5-10 yo, 20 ml ( $1\frac{1}{3}$  tablespoons) for 10-15 yo, and 40 ml ( $2\frac{2}{3}$ ) for >15 yo.
- Blood samples will be obtained in the laboratory at the same time as blood is obtained for non-research tests.
- As part of this research study, all individuals 18 and over will be tested for HIV (human immunodeficiency virus, which is the virus that causes the acquired immunodeficiency syndrome [AIDS]). You will be notified of the results of the testing, and counseled as to the meaning of the results, whether they are positive or negative. If the test indicates that you are infected with HIV, you will receive additional counseling about the significance of your care and possible risks to other people. The test results will be kept confidential to the extent permissible under the law. If you do not want to be tested for HIV, you can still take part in the study. In this case, you will receive all other research tests except for HIV.
- The results of some of the research blood tests will be available to help your doctor diagnose and treat your illness. The results of other tests, for which treatment is not routinely given and which are not routinely used or available in Viet Nam, will not be available to you and your doctor. However, these tests are new, may take months for results, and are not in routine use even overseas yet because we are learning how they work. Therefore, the research tests may be helpful in understanding causes of fever in your community and may help patients like you in the future.
- Medical information obtained for your routine care during this visit may be used in this **Research Fever study**.

**Research Procedures SECOND VISIT (2 to 4 weeks after the first visit):**

- Collect information about whether or not you got completely better
- Do a brief physical examination if needed
- Collect blood samples. Collect blood samples. The total amount of blood taken from a vein in your arm for these research tests will be about 10 to 40 ml ( $\frac{2}{3}$  to  $2\frac{1}{3}$  tablespoons); up to 10 ml ( $\frac{2}{3}$  tablespoon) for those 2-5 years old, 15 ml (1 tablespoon) for those 5-10 years old, 20 ml ( $1\frac{1}{3}$  tablespoons) for those 10-15 years old, and 40 ml ( $2\frac{2}{3}$  tablespoons) for those  $\geq 15$  years old.

Your participation for the entire study will not last more than thirty (30) days from the time of enrollment and will only include these two visits. The estimated time you spend in this study will be about two hours.



**What are the risks or discomforts of the study?**

The risks of participating in this research study should be minimal and include the following:

- Blood drawing
  - the insertion of the needle may cause temporary discomfort
  - a bruise may form at the point where the needle enters the vein.
  - in rare cases, infection or fainting may occur.

**Are there benefits to being in the study?**

- The results of all blood test drawn for the purposes of the research study that are available when you are treated will be shared with your doctor and may aid his/her decision of how best to treat you.
- If you take part in this study, you may help others in the future who develop an illness with fever in Viet Nam by allowing health care professionals to better understand the causes of disease and the use of laboratory tests for disease with fever.

**What are your options if you do not want to be in the study?**

- You do not need to participate in this study to get treatment and if you do not join, your care will not be affected. All tests that are needed for your care are available outside of the study; you will still get whatever routine tests your doctor orders.

**Will it cost you anything to be in this study?**

- Non-research tests and medical examinations are considered to be part of your routine medical care and will not be paid for by this study.
- All tests done for you and your family as part of the research study (extra blood tests and a second doctor visit) will be provided at no cost to you or the hospital.

**Will you be paid if you join this study?**

- You will be given a sum, equivalent to USD \$3, to compensate the cost of bus fare for the return visit and your time (2-3 hours of work).

**Can you leave the study early?**

- You can agree to be in the study now and change your mind later. Leaving this study early or choosing not to participate will not stop or have any effect on your regular medical care. If you wish to leave the study, please tell us right away.

**Why might we take you out of the study early?**

- You will be taken out of the study early only if we decide to end the study early.

**What other things should you know about this research study?**

- The Ethics Committees at NIHE protects the rights and welfare of people taking part in research studies. You may contact the Ethics Committee at NIHE (Chairman-telephone number) if you have questions about your rights as a participant or if you think you have not been treated fairly. You may also call these numbers for other concerns or questions about the research.

**Will the study require any of your other health care providers to share your health information with the researchers of this study?**

- As a part of this study, the researchers may ask to see your health care records from your other health care providers. We will ask these other health care providers to give us information about your recent visit for fever including history, physical exam and the results of laboratory tests, and x-rays.

**What do you do if you have questions about the study?**

- Call the principal investigator, Dr. Nguyen Tran Hien at Telephone Number (office), or email him at Email Address. If you cannot reach the principal investigator or wish to talk to someone else, call the Ethics Committee Chairman.

**What should you do if you are injured or ill as a result of being in this study?**

- Call the person in charge of this study **Dr. Nguyen Tran Hien, at Telephone Number (office), or email him at E-mail Address** if you think you experience any harm because of this study.

**Will my information be kept private?**

Study records that identify you will be kept confidential as required by law. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records. Any personal identifiers in your study records will be kept in confidential files at NIHE to be seen only by study personnel and your usual care providers. For records disclosed outside of NIHE, you will be assigned a unique code number. The key to the code will be kept in a locked file in Dr. Hien's office.

**What happens to Blood Samples collected in the study?**

- Some of your blood sample will be tested locally at NIHE and these tests will be made available to your doctor for your care. The rest of your blood sample will be sent to laboratories in the United States and Singapore to conduct tests that are not available in Viet Nam. These results will not be available for your care since the tests take time to produce results and are not fully understood now. However, the results will be available to the researchers from NIHE, HSPH, UH and Duke-NUS GMS to help us better understand the causes of illness and fever in your community. These blood samples will be retained by the current study team until testing is complete or they are used up, since the blood samples may help us develop and evaluate the best tests for rapid diagnosis of pathogens (organisms) causing fever in Viet Nam.
- Your blood sample will be identified by a code number so that your identity will remain anonymous; all identifying information from your blood sample will be removed. The only person who will have access to the identification of the code number will be Dr. Nguyen Tran Hien. He will keep the code sheet which links the sample code number with your name and hospital number in confidential files at NIHE.

**Who owns the data and blood samples that are collected in the study?**

Data records and blood specimens removed from you during the course of this study may be valuable for our current and developing understanding of causes of fever in Viet Nam. By agreeing to participate in this research, you authorize the partners in this collaboration (NIHE, HSPH, UH and Duke-NUS GMS) to use your data and blood specimens for the purpose of using existing and newer tests to evaluate the pathogens (organisms) which cause febrile illness in Viet Nam. We would not use your blood specimens for future research unrelated to this study without your consent. If at any point you decide that you do not want your data and blood samples to be used for this research, please notify Dr. Nguyen Tran Hien in writing, or email him at e-mail address. NIHE, HSPH, UH and Duke-NUS GMS will maintain these data records and blood samples until testing is complete or until the blood samples are exhausted.

**Assent Statement**

This research study has been explained to my child in my presence in language my child can understand. He/she has been encouraged to ask questions about the study now and at any time in the future.

**What does your signature/thumbprint on this consent form mean?**

You will not give up any legal rights by signing this consent form. Your signature on this form means that:

- you understand the purpose of this study, the procedures to be followed, and the risks and benefits have been explained to you
- you have been allowed to ask questions freely and have had all of your questions answered
- you know who to contact if you have additional questions
- you agree to join the study and understand that you can withdraw at any time
- you have been told that you will be given a signed copy of this consent form

**WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM**

NOT VALID WITHOUT  
THE IRB STAMP OF CERTIFICATION

**Do not sign after the expiration date of: \_\_\_\_\_**

\_\_\_\_\_  
Signature/ thumbprint of Participant for Individuals 18 & over

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Person Obtaining Consent (Must be over 18 years)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature/thumbprint of Parent/Guardian for Minors 2-18 years

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature/thumbprint of Assenting Minor between 12-18 years

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Witness to Consent Procedures  
(optional unless Ethics Board requires)

\_\_\_\_\_  
Date

**NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR; A COPY MUST BE GIVEN TO THE PARTICIPANT; AND, IF APPROPRIATE A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PARTICIPANT'S MEDICAL RECORD.**

FOR OFFICE USE ONLY:  
STUDY APPROVED FOR ENROLLMENT OF: \_\_\_\_ Adults Only \_\_\_\_ Adults and Children \_\_\_\_ Children Only

### Appendix III: Biosafety authorizations from the UH Institutional Biosafety Committee.

# *Institutional Biosafety Committee Letter of Authorization*

☒ Authorized    ☐ Approved, Pending Modification    ☐ Denied  
☒ New    ☐ Renewal    ☐ Amended

ORS Number: <b>R20062454</b>
IBC Number: <b>580B0707G</b>
IACUC Number N/A
CHS Number: N/A

Duration of Protocol: 8/1/2007 - 7/30/09

Sponsoring Agency: Dept. of Defense

☐ Prime    ☐ Sub recipient

**Title:** Advanced Technologies Addressing Asia-Pacific Infectious Disease

**Principal Investigator:** Duane J. Gubler

**NIH Classification:** Section III-F. Exempt Experiments

**Location:** Tropical Medicine, Medical Microbiology, and Pharmacology, JABSOM  
Kakaako, BSBS 320

Biosafety Level:    BSL 2, 3    Facility Inspection Date:    October 2006

Faculty Training Date: November 2006

☒ Recombinant Activities

☐ Select Agents and Biological Derived Toxins

☒ State importation

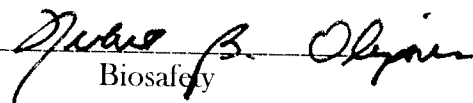
☒ Blood and Blood Products (including body fluids, cell lines, tissues)

## **ADDITIONAL CONDITONS:**

☐ Permits:

☐ Additional Comments (see attached)

  
\_\_\_\_\_  
IBC Chair  
UH IBC

  
\_\_\_\_\_  
Biosafety

Date:    AUG 2 2007

Expiration: August 2012

2040 East-West Road, Honolulu, Hawaii 96822

Telephone: (808) 956-8660, Facsimile: (808) 956-3205, Web site: <http://www.hawaii.edu/ehso/>

An Equal Opportunity/Affirmative Action Institution

# UNIVERSITY OF HAWAII

## BSP

### Biological Use Authorization and Registration

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, & human samples)

**THIS IS A:**

☒ NEW PROTOCOL

☐ REVISED PROTOCOL

☐ 5-YEAR RENEWAL

**PRINCIPAL**

**INVESTIGATOR(S):**

NAME	DEPARTMENT	BUILDING AND ROOM No.	WORK PHONE #	FAX No.
Duane Gubler	Trop. Med	BSB 320	692-1600	692-1979

Email: dgubler@hawaii.edu

**PROJECT TITLE:**

Advanced Technologies Addressing Asia-Pacific Infectious Diseases

☐ REGISTRATION INVOLVES CONFIDENTIAL INFORMATION OR INTELLECTUAL PROPERTY

☐ PERSONNEL UNDER 16 YEARS OF AGE

**OTHER INSTITUTIONAL APPROVALS:**

If you have received an approval from the appropriate review committee, please check and indicate the registration number in the corresponding box below. If you are in the process of applying to these Committees, please check "Pending".

	YES	NO	PENDING	REGISTRATION # and DATE
Vertebrate Animals (IACUC)		X		
Human Subjects (CHS)		X		

----- Do Not Write Below This Line -----

#### INSTITUTIONAL BIOSAFETY COMMITTEE ONLY

DATE RECEIVED:	
INITIAL BIOSAFETY REGISTRATION #:	5801307079

Please forward the completed forms electronically to \_\_\_\_\_  
and

Completed hardcopy forms to Biosafety Officer, Environment, Health Safety Office.

**BSP****Biological Use Authorization and Registration**

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, &amp; human samples)

**1. PROJECT/PROTOCOL TITLE:**

Advanced Technologies Addressing Asia-Pacific Infectious Diseases

**2. PROJECT FUNDING SPONSOR:**

Department of Defense

**3. GRANT PERIOD:**

8/1/2007-7/30/09

**4. PERSONNEL INFORMATION & LAB LOCATIONS:**

LIST PI(s) AND ADDITIONAL INVESTIGATORS	DEPARTMENT	TITLE	APPLICABLE TRAINING, DATES
Frederick Burkle	Public health	Professor	
Diane Taylor	Trop. Med	Professor	
Lawrence Burges	Tripler AMC	Chief	
Dr. Duane Gubler	Trop. Med	Chairman/professor	

List building(s) &amp; lab(s) where work will be conducted and date of last inspection of those facilities:

BSB 336, 303, 332 and 162 were inspected on July, 2007; Leahi Rm. 307 was inspected in October 2006.

## UNIVERSITY OF HAWAII

**Biological Use Authorization and Registration**

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, &amp; human samples)

**5. RESEARCH SUMMARY:**

Please provide a concise summary of the project in the space below. Be sure that the sources of DNA are clearly defined and the reader can determine the nature of the biological materials used (i.e., fixed, fresh, a commercial kit, replication deficient, etc.). Remember that the goal of this form is to be able to understand and assess biological risk. NOTE: This registration form may serve for more than one research project (title) if all the biological agents, personnel, and locations are the same.

Develop an active syndromic disease detection system to monitor the pathogens causing disease in humans in both urban and rural environments. A syndromic surveillance system will be established to monitor illness caused by infectious agents in the northern provinces of Viet Nam. Selected health clinics, private physicians and provincial and district hospitals will be used as sentinel sites in both urban and rural areas. Clinical samples will be taken from patients seeking medical care for fever associated illness at the sentinel sites. Use advanced technology to identify the pathogens causing illness in humans and to identify risk factors for infection. Clinical samples from patients whose illness has an unknown etiology will be sent to APITMID in Honolulu for pathogen discovery, with diagnostic testing using the latest technology, including micro-array analysis, immunohistochemistry, PCR and isolation.

**6. CLASSIFICATION (DOUBLE CLICK ON BOX TO CHECK):**

NIH Section III-A – Experiments that Require Institutional Biosafety Committee (IBC) Approval, RAC review, and NIH director approval before initiation

a) A1 - Major action under the NIH guidelines

☐ YES ☒ NO

NIH Section III-B – Experiments that Require NIH/OBA and IBC Approval Before Initiation

b) B1 - Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight

☐ YES ☒ NO

NIH Section III-C – Experiments that Require IBC and Institutional Review Board (IRB) approvals and RAC Review Before Research Participant Enrollment

c) C1 - Experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants

☐ YES ☒ NO

NIH Section III-D – Experiments that Require IBC Approval Before Initiation

d) D1 – Experiments using Risk Group (RG) 2, RG3, RG4 or restricted agents as host-vector systems

☐ YES ☒ NO

e) D2 – Experiments in which DNA is from RG 2, RG3, RG4 or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems

☐ YES ☒ NO

f) D3 - Experiments involving the use of infectious DNA or RNA viruses or defective



# UNIVERSITY OF HAWAII

## BSP

### Biological Use Authorization and Registration

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, & human samples)

DNA or RNA viruses in the presence of helper virus in tissue culture systems

☐ YES ☒ NO

g) D4 - Experiments involving whole animals

☐ YES ☒ NO

h) D5 - Experiments whole involving plants

☐ YES ☒ NO

i) D6 - Experiments involving more than 10 liters of culture)

☐ YES ☒ NO

NIH Section III-E - Experiments that Require IBC Notice Simultaneous with Initiation

j) E1 - Experiments involving the formation of recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus

☒ YES ☐ NO

k) E2 - Experiments involving whole plants

☐ YES ☒ NO

l) E3 - Experiments involving transgenic rodents

☐ YES ☒ NO

NIH Section III-F. Exempt Experiments

m) F - Exempt Experiments

*per 1/40* ☒ YES ☒ NO

n) G - Non-recombinant experiments, RG 2 or higher

☒ YES ☐ NO

o) H - Non-recombinant experiments, RG1

☐ YES ☒ NO

Please identify every biological used and the corresponding Risk Groups (RG) and Biosafety Levels (BL). Please identify all strains (purity, genotypic and phenotypic characteristics) and include ATCC or comparable information when applicable.

	SPECIFIC NAME (GENUS, SPECIES)	STRAIN	SOURCE (ATCC #, ETC.)	RG	BL
VIRUS	arbovirus		diagnostic	2,3	2,3
BACTERIA					
FUNGI					
PARASITE					
CELL LINE/ CULTURE*	Vero cells C6/36		ATCC	1	1
TOXINS					
PLANT & PLANT TISSUES					
OTHER (TISSUES, TRANSGENIC PLANTS & ANIMALS)					

\* For Cell Cultures, please list all hosts, vectors, and donor genes.

## UNIVERSITY OF HAWAII

**BSP****Biological Use Authorization and Registration**

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, &amp; human samples)

☐ Possible environmental release☐ expression of foreign gene, if any list☐ Gene therapy in animals or humans☐ Outside of the laboratory (including field trials, growth chamber, and green house). This requires the additional submission of BSP-4 Form.**7. BIOLOGICAL SUMMARY:**

This section should be used to describe containment, disposal, invasive species control, security, laboratory animal care/ethics, accidental spills & personnel contamination, and facility compliance.

BBP ECP and Biohazard waste management are all on file.

Please describe your assessment of biological hazards that a lab employee can reasonably anticipate. Are special medical surveillance practices recommended? (vaccines, respirators, etc.)  
This is required information.

These diagnostic samples are anticipated to be risk group 2 and 3. N95 will be used for risk group 3.

Please check and attach electronic copies the documents that apply. If electronic copy is not available, send hard copy to Biosafety Officer. \*mandatory

\*Emergency/contingency plans ☒  
 Permits ☒ MSDS ☐

\*Biological and sharps wastes disposal ☒  
 references ☐ product inserts ☐ SOP ☒

**8. TYPES OF ENGINEERING CONTROL:**

EQUIPMENT	TYPE/CLASS	MANUFACTURER/ MODEL	LOCATION	CERTIFICATION DATE
BIOSAFETY CABINET	Class II, type 2A and 2B	Nuare, Baker	BSB 336,162	06/14/07
LAMINAR FLOW BENCH	None			08/24/06
AUTOCLAVE	Single and Double doors	Tuttnauer	BSB 334,162	
PERSONAL PROTECTIVE EQUIPMENT	booties, gloves, tyvek suit, respirator, face shield, N 95	fisher		
DISINFECTANT (BRAND AND WORKING SOLUTION)	10% bleach and 70% Ethanol	Sigma, fisher		

## UNIVERSITY OF HAWAII

## BSP

## Biological Use Authorization and Registration

(recombinant DNA, microorganisms, cell cultures/lines, animal and plant tissues, &amp; human samples)

## 9. CERTIFICATION &amp; INVESTIGATOR SIGNATURE:

I accept responsibility for the safe conduct of work with the recombinant DNA, microorganisms, cell cultures, and/or human samples involved in this project. I have informed all personnel who may be at risk of potential exposure to these materials and have determined that the procedures to be used are appropriate for this work. I also understand that I bear the responsibility for ensuring that all personnel are adequately trained. For research that involves the use recombinant DNA, I will follow the National Institute of Health (NIH) and LBNL guidelines. For all work, I will abide by the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories" (BMBL). The information above is accurate and complete.

X Duane Gubler Principal Investigator 10 July 07 Date

----- Do Not Write Below This Line -----

## BIOSAFETY USE AUTHORIZATION (Two Options)

I certify that the Institutional Biosafety Committee (IBC) has reviewed the proposed project and has found it to be in compliance with the NIH, USDA and other applicable guidelines. The work area has been inspected. University of Hawai'i policies are being adhered to.

X James T. Douglas  
Signature of Chairperson, IBC

James T. Douglas  
PRINTED NAME of Chairperson, IBC

UH IBC  
AUG 29 2007  
Date  
EHSO BSP

Approved ☒Conditionally Approved ☐Deferred ☐

If applicable, below are the conditions for approval. You are not authorized to begin work on this project until you have satisfied the conditions listed and receive an approval form from the IBC.

OR

I certify that I have been authorized by the IBC to administratively review and approve NIH exempt and Biosafety Level 1 experiments. The proposed project has been found to be in compliance with the NIH Guidelines.

X  
Signature of Chairperson, IBC or Biosafety Officer (BSO)

Date

PRINTED NAME of Chairperson, IBC or BSO

**UNIVERSITY OF HAWAII AT MĀNOA**

Environmental Health and Safety Office

**Biological Safety Program**

November 14, 2006

**MEMORANDUM**

**TO:** Dr. Shannon N. Bennett  
T3MP - JABSOM  
BSB 336

**FROM:** Dr. James T. Douglas, Chair  
Institutional Biosafety Committee



Hubert Olipares  
Biological Safety Officer



**LETTER of AUTHORIZATION No.: 800A1006G**

**SECTION III. EXPERIMENTS COVERED BY THE NIH GUIDELINES**

E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

**APPROVED "Molecular Evolutionary Pathogenesis of Dengue Virus Infection"**

The Environmental Health and Safety Office, Biological Safety Program (BSP) and the UH Institutional Biosafety Committee (IBC), have reviewed your biological activities on Tuesday, November 14, 2006, including recombinant activities, if applicable. The IBC and BSP have approved the use as described in your protocols as submitted on the BSP-1 and 3 forms. Your laboratory at the Department of Tropical Medicine and Medical Microbiology, John A. Burns School of Medicine, Kakaako Campus, Bioscience Building, Room 336 has been certified and rated a minimum as a Biological Safety Level 2, according and as appropriate to the National Institutes of Health, Centers for Disease Control and Prevention, US Department of Agriculture (USDA) and/or as required by Hawaii Occupational Safety and Health. The biological safety guidelines for use of biological materials, including recombinant activities at the University of Hawaii are being met, to its satisfaction.

This memorandum and signed copy of your BSP-3 form is your authorization as approved by the UH IBC. Upon conducting a risk assessment of the facility, procedures, practices, training and expertise of personnel of this protocol and containment levels required by the guidelines, it meets a Biosafety Level 2. Laboratory has been inspected on September 2006.

This project conforms with UH Biosafety Guidelines as applicable to the NIH - "Guidelines for Research Involving Recombinant DNA Molecules" (DHHS, NIH April 2002), USDA (7 CFR Parts 340), and the CDC-NIH Biosafety in the Microbiological and Biomedical Laboratories (4<sup>th</sup> edition, May 1999).

All facility that houses regulated biological commodities must have a written security plan and wastes management must be properly treated (preferably autoclaved), including end of project destruction. All necessary transportation requirements must be adhered with according to US Department of Transportation. There is an UH policy on moving local isolates. Local isolates are not considered regulated biological commodities, however, standard biosafety practices (good laboratory practices) must be adhered with during active manipulation.

Remember that all imported "live biological commodities," come under the jurisdiction of State Departments of Agriculture and Health "Import and Use" permits, including, live biological materials such as *E. coli*, animals, plasmids, bacteriophage, DNA, and cell lines. All staff, faculty and students manipulating biological commodities must be initially and annually trained and documents of their training and assurances of project kept for a minimum of three years. There may be additional requirements from the (1) UH Veterinarian- Laboratory Animal Services and the Compliance Office of the Institutional Animal Care and Use Committee, (2) Committee on Human Subjects, (3) Diving Safety Control Board for scientific diving, (4) Radiation Safety Committee for radioactive usage and (5) the Workplace Safety Committee.

A final report should be provided, once the project is completed. Report immediately any injury, theft, lost, unanticipated problems and damage to the experiments or to workers. This requirement is necessary as an assurance from the National Institutes of Health and US Department of Agriculture on recombinant activities.

If there are further question or request, more information please calls me at 956-3197.

c: Karen Quinn

# BSP-1

## BIOLOGICAL USE AUTHORIZATION

☐ REGISTRATION INVOLVES CONFIDENTIAL INFORMATION OR INTELLECTUAL PROPERTY

### I. PERSONAL INFORMATION

- |   |  |
|---|--|
| 1. Principal Investigator: <u>Shannon N. Bennett</u>          |  |
| 2. Department/Unit: <u>Dept. of Tropical Medicine</u>         | 3. Campus Code: _____                    |
| 4. Building and Room No.: <u>BSB 336 (L); BSB 320D (O)</u>    | 5. Telephone No.: <u>692-1603</u>        |
| 6. Address: <u>651 Ilalo St., Kaka'ako, Honolulu HI 96813</u> | 7. Fax No.: <u>692-1979</u>              |
| 8. Granting Agency: <u>NIH/NIAD</u>                           | 9. Laboratory Telephone: <u>692-1792</u> |

### II. BIOLOGICAL COMMODITY

10. Commodity:
- |   |   |
|---|---|
| a. <input checked="" type="checkbox"/> Microorganisms             | b. <input checked="" type="checkbox"/> Medical/Clinical Specimen: _____ |
| c. <input type="checkbox"/> Plant/Plant Part                      | d. <input type="checkbox"/> Native, threatened or endangered species    |
| e. <input type="checkbox"/> Invertebrate Animal                   | f. <input type="checkbox"/> Vertebrate Animal                           |
| g. <input type="checkbox"/> Soil or Other<br>Environmental Sample | h. <input checked="" type="checkbox"/> Tissue cell line                 |
| i. <input type="checkbox"/> other (please specify): _____         |   |
11. Description: Common Name: dengue virus
12. Scientific Name: dengue type 1-4

### III. CONTAINMENT ASSESSEMENT

13. Level of Physical and Biological Containment: BSL 1 ☐ BSL 2 ☒ BSL 3 ☐  
☐ animal ☐ plant ☐ large scale
14. Types of Engineering Controls
- |   |  |
|---|--|
| a. Biological Safety Cabinet (Model, Type, Certification date):   | <u>SterilGARD ClassII Type A/B3; 12/13/2005</u>                              |
| b. Laminar Flow Clean Bench (Model, Type and Certification date): | <u>None</u>  |
| c. Autoclave (Model, Type and Certification date):                | <u>Tuttnauer Cat2007 5598SP-1V; 8/15/2005</u>                                |
| d. Personal Protective Equipment (Types):                         | <u>laboratory gown (full), face shield, gloves, closed shoes, long pants</u> |
| e. Disinfectant (Brand and Working Concentration):                | <u>Chlorox Bleach, 10%; Ethanol, 70%; Mercury RNase Free 100%</u>            |

### 15. Projected Experimental Trials:

- ☐ Field: Site Location: \_\_\_\_\_
- ☐ Greenhouse: Site Location: \_\_\_\_\_
- ☐ Screen house: Site Location: \_\_\_\_\_
- ☐ Growth Chamber Site Location: \_\_\_\_\_
- ☒ Research Laboratory Only: BSB 330 & 336
- ☐ Instructional Use Only: \_\_\_\_\_

BSP1 - Page 2 of 2

16. Describe emergency, contingency, and safety procedures: (facilities, standard operating procedures, practices and training of personnel).

- a. Biological and Sharp Wastes Disposal YES, DETAILS APPENDED
- b. Emergency/Contingency Plan: YES, DETAILS APPENDED
- c. Training (Initial, Annual, Shipping and Receiving, Bloodborne) YES, DETAILS APPENDED
- d. Other DETAILS APPENDED

17. Please attach an abstract or a description of the proposal.

To avoid unnecessary approval delays, please ensure that any narrative provides plenty of details, enough that a biomedical scientist not working in your specific field can understand your work and assess the hazards and risks. Standard procedures can be referred to by common name but novel procedures and significant modification to standard procedures should be described. Step-by-step procedures are not necessary. Do not submit other compliance committee's application or registration, no substitution, i.e., IACUC or CHS.

- a. The experimental design and goals of the research, including a brief description of the experimental procedures, please provide sufficient detail to allow the IBC to assess the hazardous potential of the experiments. (Grant or research proposal may be substituted).
- b. Containment conditions that will be implemented. The methods by which the safe conduct of the experimental procedures will be ensured.
- c. An assessment of the hazardous potential, including a brief description of the agents, it's host, modes of transmission to human, animals, or plants, and pathogenicity. Also describe the implications if the biological commodities were to be released outside of the laboratory, i.e., greenhouse.

18. Principal Investigator's Certification

- a. Ensure that listed personnel have received or will receive appropriate training in safe laboratory practice and procedures for this protocol before any work being on this project and at least annually thereafter. In addition, all listed personnel who have potential occupational exposure to biological commodities will attend an EHSO-BSP or lab specific training session.
- b. Follow the health surveillance practices as approved for this protocol and inform those working on the protocol about appropriate emergency assistance information for their location(s).
- c. Inform the BSP of any significant research, instructional, or clinical related accident or illness as soon as possible after its occurrence.
- d. Submit in writing a request for approval from the BSBP of any significant modifications to the proposal, facility, staff changes, or procedures.
- e. Adhere to the UH IBC-BSP policy, i.e., biological wastes, etc.

[Signature]  
Signature of Principal Investigator

10/25/06  
Date

INSTITUTIONAL BIOSAFETY COMMITTEE AUTHORIZATION

UH IBC

NOV 14 2006

EHSO-BSP

IBC Chair/Delegate or Biological Safety Officer: [Signature]

☒ Approve

☐ Approve with Conditions

☐ Deferred

☐ Other

BSP1

Project Description

Shannon Bennett

### Molecular Evolutionary Pathogenesis of Dengue Virus Infection.

To understand the relationship between dengue virus genotype and epidemic intensity, we will sequence virus from archived clinical samples, analyze samples for adaptive mutations, and insert these changes into an infectious clone to measure their effects on replication rate and infectivity in cell culture (various lines). Dengue viruses types 1 through 4 contained in archived sera here at Kaka'ako as well as at the Centers for Disease Control and Prevention, San Juan (the latter shipped to Kaka'ako according to BSP-2 procedures and approval) will be cultured in C6/36 (*Aedes albopictus*) cell lines, amplified by RT-PCR (both in BSB 336) and sequenced (on-campus Greenwood Molecular Biology Facility). Virus genotypes will be analyzed for adaptive changes on a gene-for-gene basis. Those genes bearing selected changes will be amplified by RT-PCR for insertion into infectious clones. Dengue infectious clones will be subjected to restriction digest to excise clone from plasmid and a restriction digest of clone using enzymes flanking the region of interest. PCR products will be similarly digested. Digested fragments will be reassembled into the plasmid using standard ligation techniques. Infectious virus will be transcribed using an in vitro transcription method based on an SP6 promoter region up stream of the insertion point in the plasmid, and used to infect various cell lines (C6/36; HepG2 [human liver]; Vero [green monkey kidney]; BHK-21 [Hamster], and *Aedes aegypti* cell lines). Replication and infectivity will be assessed using real-time RT-PCR as well as plaque-forming units in Vero cell assays.



BSP1 / BSP3

Emergency, Contingency, Safety

Shannon Bennett

**Describe emergency, contingency, and safety procedures: (facilities, standard operating procedures, practices and training of personnel).**

**a. Biological and Sharp Wastes Disposal:**

Biological waste containing virus will be soaked in a solution of 10% chlorox bleach for at least 30 minutes in the biosafety cabinet with the sash lowered, then flushed down the drain with copious amounts of running water before and after for at least 10 minutes. Containers thus emptied will be rinsed and placed in clearly labeled biological waste receptacles. Sharps that have been in contact with virus will be similarly soaked in bleach solution. The contents of the soak solution will be handled as above. The sharps will be discarded in clearly labeled sharp disposal containers. Sharps that have not been in contact with virus but other biologicals (such as media) will be discarded in clearly labeled sharp disposal containers.

**b. Emergency/Contingency Plan:**

In the event of a spill involving virus, laboratory personnel will be notified, 10% bleach solution will be applied to the area for at least 30 minutes, and the EHSO officer will be informed.

**c. Training (Initial, Annual, Shipping and Receiving, Bloodborne):**

All personnel will have taken and will be required to maintain: Lab Safety Training, Initial Biosafety Training, Annual renewal Biosafety Training, Bioshipping and Receiving, Initial/Annual High Containment Training, Hazardous Waste Generator Training, and Web CT courses Bloodborne Pathogens and Transporting and Receiving Biological Materials.

**d. Other:**

N/A

## BSP-3

# REGISTRATION OF EXPERIMENTS FOR RECOMBINANT DNA ACTIVITIES FOR LABORATORY USE

[ ] REGISTRATION INVOLVES CONFIDENTIAL INFORMATION OR INTELLECTUAL PROPERTY

1. Principal Investigator: <u>Shannon N. Bennett</u>	
2. Department/Unit: <u>Dept. of Tropical Medicine</u>	3. Campus Code: _____
4. Building and Room No.: <u>Kaka'ako; BSB 336 (L); BSB 320D (O)</u>	5. Telephone No.: <u>692-1603</u>
6. Address: <u>651 Ilalo St., Honolulu, HI 96813</u>	7. Fax No.: <u>692-1979</u>
8. Granting Agency: <u>NIH/NIAID</u>	9. Laboratory Telephone: <u>692-1792</u>

10. List of Additional Investigator (Research Associates Assistants, Graduate Students, Undergraduate Student, Staff, and other Colleagues).

Print Name(s) Clearly	Title	Signature
<u>Chase Akins</u>	<u>Technical Staff/Research Associate</u>	<u>CA</u>
<u>Alleen Duran</u>	<u>Undergraduate Research Assistant</u>	<u>AED</u>

## I. DESCRIPTION OF PROTOCOL

1. Title: Molecular Evolutionary Pathogenesis of Dengue Virus Infection
2. Protocol of Experiment: Dengue Infectious Clones: Reverse Genetics  
 Dengue infectious clones will be subjected to restriction digest to excise clone from plasmid and a restriction digest of clone using enzymes flanking the region of interest. PCR products for nonstructural gene NS2A will be similarly digested. Digested fragments will be reassembled into the plasmid using standard ligation techniques. Infectious virus will be transcribed using an in vitro transcription method based on an SP6 promoter region up stream of the insertion point in the plasmid, and used to infect various cell lines.
3. Duration of Experiment: 5 years
4. Source(s) of DNA: NIH/NIAID dengue infectious clone; original virus dengue type 4 from Dominica, 1981
5. Nature of the Inserted DNA Sequences: dengue PCR products, nonstructural genes
6. Host(s) and Vector(s) to be used: E. coli hosts plasmid vector pBR322 for clone
7. Expression of foreign gene and if any protein to be produced?: whole virus expression, chimeras of NS2A
8. Possible environmental release?: none

## II. CONTAINMENT ASSESSEMENT

1. Level of Physical and Biological Containment: Type: [ ] Animal [ ] Plant [ ] Large Scale  
 Level: [ ] BSL 1 [X] BSL 2 [ ] BSL 3

## 2. Types of Engineering Controls

- a. Biological Safety Cabinet: (Model, Type, Certification date): SterilGARD ClassII Type A/B3; 12/13/2005
- b. Laminar Flow Clean Bench (Model, Type and Certification date): None
- c. Autoclave (Model, Type and Certification date): Tuttnauer Cat2007 5598SP-1V; 8/15/2005
- d. Personal Protective Equipment (Types): laboratory gown (full), face shield, gloves, closed shoes, long pants
- e. Disinfectant: (Brand and Working Concentration): Chlorox Bleach, 10%; Ethanol, 70%; Mercury RNase Free

## 3. Projected Experimental Trials:

- ☐ Field Trial: Site Location: \_\_\_\_\_
- ☐ Greenhouse: Site Location: \_\_\_\_\_
- ☐ Screen house: Site Location: \_\_\_\_\_
- ☐ Growth Chamber Site Location: \_\_\_\_\_
- ☒ Research Laboratory Only: BSB 330 & 336
- ☐ Instructional Use Only: \_\_\_\_\_

## 4. Describe emergency, contingency, and safety procedures: (facilities, standard operating procedures, practices and training of personnel).

- a. Biological and Sharp Wastes Disposal:  
YES, DETAILS APPENDED
- b. Emergency/Contingency Plan:  
YES, DETAILS APPENDED
- c. Training (Initial, Annual, Shipping and Receiving, Bloodborne):  
YES, DETAILS APPENDED
- d. Other: DETAILS APPENDED

I agree to (1) comply with the National Institutes of Health (NIH), US Department of Agriculture-Animals, Plant Health Inspection Services (USDA-APHIS), requirements pertaining to shipment and transfer of recombinant materials, (2) I am familiar with the provisions of the current NIH and USDA Guidelines and other specified federal, state, and municipal rules and regulations pertaining to the recombinant materials, (3) inform the Institutional Biosafety Committee (IBC) if there are any amendments to the proposal and (4) allow the IBC/Biological Safety Officer (BSO) access to the work area to check for compliance with governmental regulations. I believe the above information is accurate and complete.

Principal Investigator

Date: \_\_\_\_\_

## INSTITUTIONAL BIOSAFETY COMMITTEE AUTHORIZATION

I certify that the Institutional Biosafety Committee has reviewed the proposed recombinant project and has found it to be in compliance with the NIH, USDA and other applicable Guidelines. The work area has been inspected. University of Hawaii's policies are being adhered to.

IBC Chair/Designee or BSO

Date: \_\_\_\_\_

NOV 14 2006

EHSO BSP

☒ Approved☐ Approved with Conditions☐ Deferred

BSP1 / BSP3

Emergency, Contingency, Safety

Shannon Bennett

**Describe emergency, contingency, and safety procedures: (facilities, standard operating procedures, practices and training of personnel).**

**a. Biological and Sharp Wastes Disposal:**

Biological waste containing virus will be soaked in a solution of 10% chlorox bleach for at least 30 minutes in the biosafety cabinet with the sash lowered, then flushed down the drain with copious amounts of running water before and after for at least 10 minutes. Containers thus emptied will be rinsed and placed in clearly labeled biological waste receptacles. Sharps that have been in contact with virus will be similarly soaked in bleach solution. The contents of the soak solution will be handled as above. The sharps will be discarded in clearly labeled sharp disposal containers. Sharps that have not been in contact with virus but other biologicals (such as media) will be discarded in clearly labeled sharp disposal containers.

**b. Emergency/Contingency Plan:**

In the event of a spill involving virus, laboratory personnel will be notified, 10% bleach solution will be applied to the area for at least 30 minutes, and the EHSO officer will be informed.

**c. Training (Initial, Annual, Shipping and Receiving, Bloodborne):**

All personnel will have taken and will be required to maintain: Lab Safety Training, Initial Biosafety Training, Annual renewal Biosafety Training, Bioshipping and Receiving, Initial/Annual High Containment Training, Hazardous Waste Generator Training, and Web CT courses Bloodborne Pathogens and Transporting and Receiving Biological Materials.

**d. Other:**

N/A

Appendix IV: Protocol translated to Vietnamese.



1. Thu thập 1.000 mẫu huyết thanh bệnh nhân sốt không rõ nguyên nhân.
2. Sưng lách lâm tại trở hai bệnh: Sốt Dengue/Sốt xuất huyết Dengue và Viêm não Nhật Bản B bằng kỹ thuật MAC-ELISA
3. Các mẫu huyết thanh tìm thấy âm tính gửi sang Hoa Kỳ để tìm ra căn nguyên
4. Xảy đúng một hồ thành thử điểm chỉ định chính báo để theo dõi vụ phát hiện sớm các mầm bệnh truyền nhiễm cho người tại Thành phố và vùng Thôn của miền Bắc Việt Nam. Xác định căn nguyên gây nên hội chứng sốt không qua số đông các kỹ thuật mới nhất.

**1 Tầm nhìn nghiên cứu trong vụ ngoại n-ic**

Tầm nhìn tổng thể

☒ Mới

**Tầm nhìn nghiên cứu ở n-ic ngoại các bệnh Sốt Dengue, Viêm não, Bệnh viêm màng não, Bệnh có gia cầm H5N1, Bệnh sốt xuất huyết do virus Hantaan và các sốt khác**

Trong vòng 15 năm, nhiều dịch bệnh nguy hiểm ở khu vực công nhân tập thể xảy ra và lớn ở các tỉnh phía nam gây ảnh hưởng lớn đến nền kinh tế xã hội. Tất cả các bệnh dịch nguy hiểm này (như dịch hạch ở Ấn Độ vào năm 1994, dịch có gia cầm tại Hồng Kông 1997, dịch viêm não Nipah tại Malaysia, dịch SARS hay hội chứng hô hấp cấp tính ở Trung Quốc từ năm 2002 đến 2003, vụ có gia cầm ở vùng Đông Nam, từ năm 2003 đến 2007)... đều có mầm bệnh từ động vật phát sinh tại. Chưa vụ dịch vụ lây lan làm ảnh hưởng đến nền kinh tế xã hội. Vì vậy trong tương lai tập thể họ, hiện nay, chúng ta cần thiết phải chú trọng lại sẽ xảy ra nhiều dịch bệnh ở các mức độ khác nhau. Hơn nữa, do các yếu tố xã hội, văn hóa, và nhân khẩu ở châu... cả lý vấn lại tiếp tục xuất hiện các dịch bệnh bất ngờ nguồn gốc động vật... Việc phát triển một chương trình "chiến lược dự phòng sớm" tại châu sẽ giúp chúng ta chủ động phát hiện và ngăn chặn kịp thời các bệnh dịch để biết đến cùng nhau các bệnh dịch mới phát sinh.

**A. Sốt Dengue/ Sốt xuất huyết Dengue- SD/ SXHD  
(Dengue Fever/ Dengue Haemorrhagic Fever- DF/ DHF)**

Sốt Dengue/ sốt xuất huyết Dengue (SD/ SXHD) là một bệnh nhiễm trùng cấp tính do virus dengue gây ra. Bệnh không có vắc xin và thuốc điều trị. Biểu hiện lâm sàng của SD/ SXHD rất đa dạng từ sốt cao đột ngột kéo dài 2-7 ngày kèm theo triệu chứng đau đầu, đau cơ, đau khớp, đau bụng cho tới buồn nôn, phát ban... Sau đó cả triệu chứng xuất huyết da, xuất huyết nội tạng (chảy máu cam, chảy máu mũi, nôn ra máu, đi ngoài ra máu...). Người ra cần cả triệu chứng để chẩn đoán (Barnes W. J. S. và Rosen L., 1974). Bệnh SD/ SXHD lây bệnh do muỗi (*Aedes aegypti*, *Aedes albopictus*) truyền và về bệnh thường do muỗi phát triển thành dịch. Virus Dengue âm tính Sabin phân lập ở Ấn Độ, Ấn Độ, New-Guinea và Hawaii

(Sabin A. vụ cs, 1952). Sau này @-íc x.c @Pnh lụ virut Dengue type 1-Hawaii vụ virut Dengue type 2-New-Guinea. C.c type virut Dengue 3 vụ virut Dengue 4 @-íc Hammon W.M. vụ cs phòn lệp ở Philippines vào n"m 1956 (Hammon W.M. vụ cs 1960). Tiỏp @ã nhiều chủng virut Dengue @. @-íc phòn lệp tở nhiều vũng kh.c nhau tr"n thỏ giớ nh-ng @òu @-íc x.c @Pnh lụ thuốc 4 type huyết thanh n"u tr"n (Anonymous, 1986).

Túi n"m 1997 bởnh SD/ SXHD @. lan rẻng tr"n ph"m vi t"san thỏ giớ. Theo Gubler, D.J. (1997) thx hiỏn nay cả túi h-n 2,5 tũ ng-êi @ang sẻng trong khu vùc cả l-u hủnh SD/ SXHD vụ hủnh n"m cả kho"ng 100 triỏu ng-êi m%c bởnh này. Số cả thỏ ph"ng chẻng bởnh mét c, ch hiỏu qu" thx viỏc ch" óẻng gi, m s, t huyết thanh hắc, d"ch tở hắc, c"ng tr"ng hắc virut Dengue g"y n"n bởnh sẻt Dengue/sẻt xuẻt huyết Dengue lụ hỏt sỏc c"n thiỏt. Viỏc ph, t hiỏn nhanh virus Dengue s" giỏp cho c, c b, c sũ lỏm sủng cả h-íng @iỏu tr"p th"ch hủp vụ cả hiỏu qu", lủm gi"m tũ lỏ m%c vụ tũ lỏ chỏt vx bởnh này.

Cả nhiều ph-"ng ph, p chẻn @o, n sẻt Dengue/ sẻt xuẻt huyết Dengue trong ph"ng th"y nghiỏm, tuy nhi"n @òu dũa vào nguy"n t%c:

- Ph, t hiỏn kh, ng thỏ (IgG, IgM) kh, ng virut Dengue trong huyết thanh bởnh nhỏn.
- Ph, t hiỏn virut trong huyết thanh ho"c trong c, c tẻ chỏc cả kh" n"ng nghiỏm virut.

C, c ph-"ng ph, p ch" yỏu chẻn @o, n huyết thanh hắc của virut Dengue bao g"m: Ph"n ẻng Ng"n ng-ng kỏt h"ng c"u (HI), ph"n ẻng kỏt hủp bẻ thỏ (CF), ph"n ẻng trung h"sa gi"m @, m ho"i tở (PRNT), ph"n ẻng miỏn d"ch hẻp phỏ g"n enzym ph, t hiỏn kh, ng thỏ IgM (MAC-ELISA) vụ ph"n ẻng miỏn d"ch hẻp phỏ g"n enzym ph, t hiỏn kh, ng thỏ IgG (GAC-ELISA), kũ thuẻt RT- PCR

### **1. Ph"n ẻng Ng"n ng-ng kỏt h"ng c"u (Hemagglutination Inhibition Tests- HI).**

S"y lụ kũ thuẻt cẻ @iỏn nhẻt, @-íc sỏ dỏng rẻng r"i trong chẻn @o, n huyết thanh hắc kh"ng ph"i chỏ ri"ng @ẻi vủ virut Dengue mụ c"n cho nhiều lo"i virut cả kh" n"ng ng-ng kỏt h"ng c"u n"i chung. Kh, ng nguy"n đ"ng trong ph"n ẻng HI @ó chẻn @o, n huyết thanh hắc của sẻt Dengue/ sẻt xuẻt huyết Dengue lụ kh, ng nguy"n ng-ng kỏt h"ng c"u (HA) th-ẻng @-íc s"n xuẻt tr"n n-o chẻt b"ch mủ nẻ vụ tr"n nu"i tở bủ. Kh, ng thỏ HI t"n t"i rẻt lỏu trong huyết thanh bởnh nhỏn vx vẻy ph"n ẻng Ng"n ng-ng kỏt h"ng c"u cỏng lụ kũ thuẻt th-ẻng @-íc đ"ng trong viỏc @iỏu tra d"ch tở huyết thanh hắc của virut Dengue (Clark, D. H. vụ Casals, 1958). Tuy nhi"n kũ thuẻt HI chỏ x, c @Pnh @-íc sũ nghiỏm virut Dengue n"i chung chỏ kh" cả thỏ kỏt luẻn @-íc bởnh nhỏn nghiỏm virut thuốc typ nủ. Ngo"i ra, trong chẻn @o, n huyết thanh hắc b"ng ph"n ẻng HI c"n ph"i cả m, u kỏp (mẻt 2-3 tũc) @ó @, nh gi, óẻng lúc kh, ng thỏ vx thỏ kỏt qu" th-ẻng rẻt chẻm (Gubler, D.J. vụ Clark, G.G., 1995).

### **2. Ph"n ẻng trung h"sa gi"m @, m ho"i tở (Plaque Reduction Nutralization Test- PRNT).**

Nh"m ph, t hiỏn kh, ng thỏ trung h"sa virut Dengue trong huyết thanh bởnh nhỏn m%c SD/ SXHD. S"y lụ ph"n ẻng nh"y vụ @"c



hiệu, cả ý nghĩa trong việc định giá, khi năng lực miễn dịch của cơ thể chống lại virus Dengue sau nhiễm trùng từ nhiễm hoặc gây miễn dịch thực nghiệm (Russell, P.K. và Nisalak, A., 1987). Khi,ng thối trung hầu như rất lâu và vậy sau khi nhiễm trùng hoặc gây miễn dịch chỉ đáng, cơ thể sẽ cả miễn dịch lâu dài đối với virus Dengue cũng vậy. Điều này cũng cả ý nghĩa rất lớn đối với việc điều tra hải cầu huyết thanh học của virus Dengue trên cơ thể sẽ khi,ng thối trung từ 1-2. Tuy nhiên, PRNT là phần ông rất tiên, phức tạp và đòi hỏi trang thiết bị phức tạp nên việc sử dụng phương pháp này trong các phòng thí nghiệm vẫn còn nhiều hạn chế (Hayes, E.B. và Gubler, D.J., 1992).

### 3. Kỹ thuật miễn dịch hấp phụ gắn enzyme phát hiện khi,ng thối IgM

**(IgM Capture Enzyme- Linked Immunosorbent Assays- MAC-ELISA).** Trong những năm gần đây, kỹ thuật này được sử dụng rộng rãi cho việc chẩn đoán sớm virus Dengue. Khi,ng thối thuốc lớp IgM hình thành sớm hơn khi,ng thối thuốc lớp IgG và tồn tại trong huyết thanh một thời gian ngắn (trong vòng 1-3 tháng) và cho cả hiệu giá, cao ở các trường hợp nhiễm trùng tiên phát (Kuno, G., và cs, 1991). Chính vì vậy MAC-ELISA rất cả ý nghĩa trong việc định giá, nhiễm trùng huyết từ của virus Dengue và đặc biệt tiên lợi so với các phương pháp chẩn đoán huyết thanh học và cho cần lấy 1 mẫu máu và sau 5 giờ sẽ cho kết quả với độ chính xác 75%- 80% (Kuno, G., và cs, 1998).

### 4. Kỹ thuật miễn dịch hấp phụ gắn enzyme phát hiện khi,ng thối IgG

**(IgG Capture Enzyme- Linked Immunosorbent Assays- GAC-ELISA).** Đây là một kỹ thuật ELISA thường được dùng để phát hiện khi,ng thối chống virus Dengue thuốc lớp IgG. Phương pháp này cả nguy cơ lý do nên giảm lượng gắn bản bằng khi,ng nguy cơ tạo thành phát hiện khi,ng thối chống khi,ng nguy cơ đã, thường thành công ta gắn bản với khi,ng nguy cơ HA tinh chế. Kỹ thuật này như hơn HI rất nhiều, tuy nhiên khi,ng đặc hiệu nên cũng cho cả thối định giá, nhiễm virus Dengue cho khi,ng thối nên biết để nhiễm typ nào. Thời gian thực hiện từ 6- 8 giờ (Chungue, E. và cs, 1989).

### 5. Phần ông đặc biệt chuyên Polymerase-phiên mã ngược

**(RT- PCR, Reverse Transcription- Polymerase Chain Reaction).** Phần ông đặc biệt chuyên polymerase (PCR) được Kary Mullis phát minh năm 1983 và công bố rộng rãi vào các năm 1986, 1987 (Mullis K. và cs 1986, Mullis K. và Faloona F, 1987), được coi là một trong số các kỹ thuật sinh học phân tử vĩ đại nhất của thế kỷ 20. Khi,ng thối kó hốt được các công trình công bố thuốc nhiều lĩnh vực khác nhau và số đông kỹ thuật này trong đã cả chẩn đoán và phân typ các tác nhân gây bệnh. Tuy nhiên đối với các tác nhân gây bệnh cả vết liệu di truyền là ARN trong đã cả virus dengue thành cần cả biến chuyển từ ARN sang cDNA, và vậy được ông đồng thuận hơn. Tuy vậy, ngay những năm 1990, kỹ thuật PCR được ông đồng rộng rãi trong chẩn đoán và phân typ virus dengue (Henchal EA và cs, 1991; Lanciotti RS và cs, 1992; Maneekarn N và cs, 1993 v.v...). Cho đến nay, PCR vẫn là kỹ thuật rất hiệu quả và được ông đồng rộng rãi trong chẩn đoán và phân typ virus

dengue (De Paula SO vụ cs, 2004; Lemmer K vụ cs, 2004 v.v...).

C.kỹ thuật trên cho thấy:

- Số chính xác từ 75% đến 80%.
- Số nhạy từ 70% đến 87%.
- Thời gian th-êng từ 4-5 giờ (MAC-ELISA) đến trên 2 tuần (HI)
- Trang thiết bị rất tốn
- Qu. trình thao t.c phải đặc thù hiển trong Phòng thí nghiệm chuẩn thực.

**B. Bệnh viêm đường hô hấp cấp.  
(Severe Acute Respiratory Syndrome- SARS)**

Hội chứng viêm đường hô hấp cấp tính nặng (SARS) là một bệnh dịch mới xuất hiện trên phạm vi toàn cầu. SARS do xuất hiện tại 23 Quốc gia: Trung Quốc, Hồng Kông, Singapore, Canada, Hoa Kỳ, Việt Nam, Malaysia, Thái Lan... Tính đến tháng 04 năm 2004 trên toàn thế giới có cả tổng số mắc/ tổng số chết là: 3169/ 144 người (Theo Tổ chức Y tế Thế giới- WHO).

T.c nhân gây bệnh SARS là một biến thể của Corona (Coronaviridae); Một nhóm virus có vỏ bọc bệnh cho cả người và động vật. Trên người, Corona th-êng gây ra viêm đường hô hấp trên, chủ yếu người trẻ em. Ngoài ra còn cần chú ý tới một số loại vi khuẩn, virus có thể th-êng xuyên cả mặt ở đường hô hấp của người; Cả vỏ bọc gặp phải gây ra các biến chứng đường hô hấp dưới và viêm phổi nặng khác biệt trên bệnh nhân SARS.

Virus này có sức đề kháng yếu, tồn tại với nồng độ cao trong không khí ẩm, từ 2- 5 giờ. Virus SARS nhạy cảm với nhiệt độ cao, tia cực tím, các hóa chất khử trùng...

Nguồn bệnh và chứa virus SARS: Chưa biết rõ ràng. Người cả vỏ bọc nguồn bệnh chính trong chuỗi mắc lây truyền Người- Người. Người bệnh SARS, nhất là bệnh nhân nặng đang trong giai đoạn khởi phát và toàn phát là nguồn truyền nhiễm nguy hiểm. Theo qui luật chung của các bệnh do virus thì SARS cả vỏ bọc mắc bệnh từ 5 đến 15 ngày sau khởi phát.

Thời gian ủ bệnh trung bình 7 ngày; Một số trường hợp cả vỏ bọc dài tới 14- 15 ngày.

Triệu chứng: Hết h-ít, chảy nước mắt, ngứa mắt, ho, đau họng hoặc khô họng... Cả vỏ bọc đến biến chứng như: Nhiễm khuẩn huyết, viêm màng não do bội nhiễm vi khuẩn...

Kỹ thuật chẩn đoán trong Phòng thí nghiệm:

1. Phát hiện trực tiếp virus hoặc kháng nguyên virus. Cả vỏ bọc kết quả trong vài giờ nếu bệnh phẩm lấy tét; Rải trên hình các kỹ thuật sau:
  - Kỹ thuật Miễn dịch phát huỳnh quang gián tiếp là phương pháp nhạy cảm có phát hiện virus trong mẫu bệnh phẩm lâm sàng và

nuôi cấy tế bào. Tỷ lệ d- $\gamma$ ng tỷ lệ 30%.

- Các phương pháp khác: Phản ứng chuỗi polymerase (PCR) đang trong nghiên cứu. Kỹ thuật này khi chuyển xác định bằng các trang bị kỹ thuật rất tiên tiến cũng như tay nghề của các kỹ thuật.

## **2. Chẩn đoán huyết thanh học. (DFA- Direct Fluorescent Antibody- Phương pháp trực tiếp)**

### **Kỹ thuật ELISA (Enzyme- Linked Immunosorbent Assays).**

Sơ lược phương pháp miễn dịch Enzyme phát hiện kháng nguyên bằng cách các kháng thể gắn vào giá đỡ. Giá đỡ là các giếng của tấm nhựa vi lỏng hay các hạt plastic nhỏ. Cho bệnh phẩm (kháng nguyên) vào các giếng (cả giếng kháng thể trước giá đỡ). Sau đó kháng nguyên - kháng thể - $\gamma$  phát hiện bằng cách hấp trực tiếp với Enzyme. Các phương pháp giá đỡ tiếp, kháng thể kháng - $\gamma$  - $\gamma$  đều mang - $\gamma$  phát hiện bằng kháng thể kháng IgG đặc hiệu loại cách hấp với Enzyme. Enzyme gắn sau đó - $\gamma$  phát hiện và phản lỏng bằng sử dụng màu hoặc phát quang khi bổ sung cơ chất - $\gamma$ ng. Kết quả đặc trưng màu và phản ứng mét các chất quan trọng vi tính.

## **3. Kỹ thuật sinh học phân tử phát hiện virus SARS**

### **3.1. Phản ứng dây chuyền polymerase phiên mã ngược (RT- PCR- Reverse Transcription- Polymerase Chain Reaction)**

Sau khi phân lập, ngay từ những tháng đầu tiên của năm 2003, genome của virus SARS - $\gamma$  - $\gamma$  nhiều phản ứng thử nghiệm thuốc các nhà khoa học nhau trên thế giới như Trung Quốc (Qin, E., và cs, 2003), Italia (Balotta, C., và cs, 2003), CH liên bang Nga (Onishchenko, G.G., và cs, 2003), Singapore (Ruan, Y., và cs, 2003), Đài Loan (Yang, J.Y., và cs, 2003) v.v... giới m. Genome của virus SARS là ARN sợi đơn d- $\gamma$ ng với độ dài hơn 29.700 bp. Sau khi - $\gamma$  biết - $\gamma$  - $\gamma$  trình tự gen của virus SARS, các cấp mã - $\gamma$  - $\gamma$  thiết kế bộ phát hiện virus này trong bệnh phẩm bằng RT-PCR. Rất nhiều công trình - $\gamma$  công bố về việc ứng dụng RT-PCR để chẩn đoán SARS (Houng HS, và cs, 2004; Poon LL, và cs, 2004; Grant PR, và cs, 2003; Guan Y, và cs, 2003) v.v...

### **3.2. Kỹ thuật real-time PCR chẩn đoán SARS**

Kỹ thuật real-time PCR là kỹ thuật nhanh và chuẩn xác, kháng nguyên phản ứng mang phản lỏng - $\gamma$  - $\gamma$  t, c như gây bệnh và - $\gamma$  - $\gamma$  ứng dụng trong chẩn đoán nhiều bệnh khác nhau. Tuy nhiên sơ lược kỹ thuật rất tiên tiến và yêu cầu thiết bị hiện đại. Kỹ thuật real-time PCR - $\gamma$  - $\gamma$  sử dụng trong chẩn đoán SARS đầu tiên vào tháng 12 năm 2003 (Lau LT, và cs, 2003; Poon LL, và cs, 2003; Grant PR, và cs, 2003 v.v...). Trong năm 2004, nhiều công trình ứng dụng real-time PCR để chẩn đoán SARS vẫn tiếp tục - $\gamma$  - $\gamma$  công bố (Emery SL, và cs, 2004; Hourfar MK, và cs, 2004; Jiang SS, và cs, 2004 v.v...). .

## **C. Có thể gia cầm sang - $\gamma$ - $\gamma$**

Virus có thể họ Orthomyoviridae, có 3 typ: Cúm A, B và C. Trong đó cúm A B gây bệnh cho người. Cấu trúc phân tử của virus cúm: Capsid HA và NA; Màng lipid, và capsid. Vỏ lõi di truyền là 8 phân đoạn ARN sợi đơn mã hóa cho 7 protein cấu trúc và 3 protein phi cấu trúc.

- Cúm A gây nhiễm cho: người, chim, lợn, ngựa, hải cẩu, các voi và các động vật khác... Chim hoang dã là vật chủ của loài

virut nay. Cóm A chia ra hai phøn tít dùa vao 2 protein trªn bô mæt h¹t virut. Phøn tít cóm A ®-íc ®æt tªn theo c,c protein bô mæt HA vµ NA của chõng. Ví dô Virus cóm H5N1 lµ cả HA 5 protein vµ NA la 1 protein

- Cóm B th-êng gæp ẽ ng-êi nh-ng ch-a gøy nªn ®¹i dõch nh-cóm A.

- Cóm C gøy bõnh nhñ ẽ ng-êi vµ kh«ng gøy thụnch dõch

- Chõng virus: cóm B vµ phøn tít cóm A ®-íc ®æc tr-ng ẽ tªn chõng; Cả nhiõu chõng cóm B vµ phøn tít cóm A. C,c chõng cóm mõi xuýt hiõn ®ó thay thõ c,c chõng cò. Qu, trªnh nuy xªy ra khi thay ®æi tít, gãi lµ hiõn t-ìng trªi kh,ng nguyªn (drift) vµ tr-ít kh,ng nguyªn (shift). Mæt khi xuýt hiõn chõng cóm mõi ẽ ng-êi, kh,ng thõ xuýt hiõn sau khi ®. nhiõm virus cóm mõi.

Virut cóm ng-êi kh,c virut cóm gia cçm: Ng-êi cả thõ nhiõm virut cóm A, B vµ C; Tuy nhiªn chõ cả phøn tít cóm A th-êng gøy bõnh cho ng-êi la A/H1N1, A/ H3N2. Trong khi ®ã chõ cả virus cóm A gøy nhiõm cho chim. Chim nhiõm virut nh-ng kh«ng m¼c bõnh ®iõn hªnh; Cõn c,c lãai gia cçm khi nhiõm l¹i cả triõu chõng ®iõn hªnh dãn ®õn chõtt.

Virus cóm cả thõ biõn ®æi b»ng nhiõu c, ch...Mæt tít ®-íc gãi lµ trªi kh,ng nguyªn cả nghũa lµ sù biõn ®æi xªy ra ýt mæt vµ liªn tõt trong mæt thêi gian ãi...Sªn phøm của virus biõn ®æi cả thõ kh«ng nhẽn thêy bẽi kh,ng thõ t¹o ra sím h-n chõng virus cóm. Mæt tít kh,c ®-íc gãi lµ tr-ít kh,ng nguyªn hay ®æi chõ kh,ng nguyªn (antigene shift). Hiõn t-ìng tr-ít kh,ng nguyªn ®ét ngét th-êng thêy ẽ cóm A. Kõtt quª lµ mæt virus mõi hªnh thụnch, gøy bõnh cho ng-êi vµ cả protein HA hoæc cª HA vµ NA mõi ®-íc ph,t hªªn ẽ ng-êi. Nõu mæt phøn tít tít mõi hªnh thụnch xõm nhẽp vao quçn thõ ãon c- mụ cãng ®ãng ãon c- cả ýt hoæc kh«ng cả khª nªng bªo võ thª sĩ xªy ra ®¹i dõch

Tæt cª c,c virus cóm A ®õu gøy bõnh cho gia cçm. Cóm A H5 vµ H7 cả thõ phøn biõtt ®-íc ãng bõnh lý nªng hay nhñ trªn c- sẽ ãi truyõn hãc của virus vµ tsinh trçm trãng của bõnh ẽ gia cçm. Cả ba phøn tít ®iõn hªnh của virut cóm gia cçm:

- . Influenza A/H5
- . Influenza A/H7
- . Influenza A/H9

Sù løy lan virus cóm gia cçm trong c,c lãai chim, gụ vªt: Mæt sè lãai thõy cçm ®ãng vai trõ quan trãng trong viõc løy lan virus cóm. Chõng mang virus ẽ ®-êng tiªu hãa vµ ®uõ thªi ra ngoµi qua dõch mõi hãng, phøn...

Cóm A løy tã ®éng vết sang ng-êi: C,c phøn tít gøy dõch ẽ ng-êi lµ: H3N2, H2N2, H1N1, H1N2...Cóm gụ løy sang ng-êi b»ng hai c, ch: Truç tiõp tã chim, gụ, vªt...vµ qua vết chñ trung gian nh- lĩn. Virus cả 8 ®ãan gen riªng biõtt; c,c ®ãan gen cý thõ l¼p r,p kh,c nhau vµ t¹o thụnch mæt virus cóm A mõi. Kõtt quª lµ virus mõi cả thĩ gøy bõnh cho ng-êi vµ truyõn tã ng-êi sang ng-êi nh-ng nã phªi cả kh,ng nguyªn bô mæt HA vµ NA kh«ng tãm thêy tr-íc ®ã ®. nhiõm cho ng-êi. Sù biõn ®æi nuy gãi lµ "hiõn t-ìng trªi kh,ng nguyªn"

D. Bõnh sèt xuýt huyõtt do virus Hantaan gøy nªn  
- Tªnh hªnh nghiªn cõu ngoài n-íc.



- T<sup>x</sup>nh h<sup>x</sup>nh nghi<sup>n</sup> c<sup>o</sup>u trong n-íc  
T<sup>i</sup>i Vi<sup>o</sup>t Nam, h<sup>c</sup>u nh- r<sup>ê</sup>t Y<sup>t</sup> ð<sup>o</sup> t<sup>u</sup>i nghi<sup>n</sup> c<sup>o</sup>u nhi<sup>o</sup>m Hantaan virus; C<sup>o</sup>n nghi<sup>n</sup> c<sup>o</sup>u v<sup>o</sup> gien c<sup>n</sup>a virus Hantaan g<sup>o</sup>y n<sup>n</sup> b<sup>o</sup>nh s<sup>ê</sup>t xu<sup>ê</sup>t huy<sup>o</sup>t do virus Hantaan thi h<sup>o</sup>n t<sup>o</sup>n ch- a c<sup>a</sup> c<sup>o</sup>ng tr<sup>x</sup>nh n<sup>u</sup>o...

Trong nh<sup>o</sup>ng n<sup>o</sup>m g<sup>c</sup>n ð<sup>o</sup>y, t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh V<sup>u</sup>nh Ph<sup>o</sup>, H<sup>o</sup>u B<sup>x</sup>nh, H<sup>u</sup> T<sup>o</sup>y, Thanh H<sup>o</sup>, Th<sup>i</sup> B<sup>x</sup>nh... xu<sup>ê</sup>t hi<sup>o</sup>n nhi<sup>o</sup>u b<sup>o</sup>nh nh<sup>o</sup>n s<sup>ê</sup>t v<sup>i</sup>i tri<sup>o</sup>u ch<sup>o</sup>ng l<sup>o</sup>m s<sup>u</sup>ng g<sup>c</sup>n gi<sup>e</sup>ng v<sup>i</sup>i b<sup>o</sup>nh c<sup>o</sup>nh c<sup>n</sup>a S<sup>ê</sup>t Dengue/ S<sup>ê</sup>t xu<sup>ê</sup>t huy<sup>o</sup>t Dengue, nh- ng kh<sup>o</sup>ng ph<sup>t</sup> hi<sup>o</sup>n ð-íc kh<sup>o</sup>ng th<sup>o</sup> IgM v<sup>u</sup> IgG kh<sup>o</sup>ng virus Dengue, k<sup>o</sup>t qu<sup>u</sup> ph<sup>o</sup>n l<sup>ê</sup>p t<sup>o</sup> b<sup>u</sup>o c<sup>o</sup>ng cho k<sup>o</sup>t qu<sup>u</sup> c<sup>o</sup>m t<sup>y</sup>nh. S<sup>o</sup> t<sup>u</sup>i c<sup>ê</sup>p B<sup>é</sup> "B-íc ð<sup>o</sup>u ph<sup>t</sup> hi<sup>o</sup>n nhi<sup>o</sup>m Hantaan virus tr<sup>n</sup> qu<sup>c</sup>n th<sup>o</sup> b<sup>o</sup>nh nh<sup>o</sup>n t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh ð<sup>o</sup>ng b<sup>o</sup>ng mi<sup>o</sup>n B<sup>o</sup>c Vi<sup>o</sup>t Nam, 1998-2000" ð<sup>o</sup> ph<sup>t</sup> hi<sup>o</sup>n Hantaan virus tr<sup>n</sup> qu<sup>c</sup>n th<sup>o</sup> b<sup>o</sup>nh nh<sup>o</sup>n v<sup>u</sup> chu<sup>ê</sup>t t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh tri<sup>o</sup>n khai ð<sup>o</sup> t<sup>u</sup>i nghi<sup>n</sup> c<sup>o</sup>u. Theo k<sup>o</sup>t qu<sup>u</sup> c<sup>n</sup>a ð<sup>o</sup> t<sup>u</sup>i, trong n<sup>o</sup>m 1998-1999, b<sup>o</sup>ng k<sup>u</sup> thu<sup>ê</sup>t ng- ng k<sup>o</sup>t h<sup>o</sup>ng c<sup>o</sup>u th<sup>o</sup> ð<sup>é</sup>ng, ð<sup>o</sup> ph<sup>t</sup> hi<sup>o</sup>n 4,84% (12/ 248) c<sup>c</sup> m<sup>ê</sup>u huy<sup>o</sup>t thanh ng-êi v<sup>u</sup> 7,4% (2/ 27) c<sup>c</sup> m<sup>ê</sup>u huy<sup>o</sup>t thanh chu<sup>ê</sup>t d- ng t<sup>y</sup>nh v<sup>i</sup>i Hantaan virus. N<sup>o</sup>m 2000, ph<sup>t</sup> hi<sup>o</sup>n 10,24% c<sup>c</sup> m<sup>ê</sup>u huy<sup>o</sup>t thanh ng-êi d- ng t<sup>y</sup>nh (21/ 225).

Nh- v<sup>ê</sup>y, Hantaan virus c<sup>a</sup> m<sup>ê</sup>t t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh mi<sup>o</sup>n B<sup>o</sup>c Vi<sup>o</sup>t Nam. Vi<sup>o</sup>c tri<sup>o</sup>n khai nghi<sup>n</sup> c<sup>o</sup>u ð<sup>o</sup>y ð<sup>o</sup>ñ v<sup>o</sup> c<sup>ê</sup>u tr<sup>o</sup>c gien, t<sup>x</sup>nh h<sup>x</sup>nh d<sup>ê</sup>ch t<sup>o</sup> h<sup>o</sup>c, ð<sup>é</sup>ng v<sup>ê</sup>t h<sup>o</sup>c v<sup>u</sup> huy<sup>o</sup>t thanh h<sup>o</sup>c nhi<sup>o</sup>m virus Hantaan l<sup>u</sup> h<sup>o</sup>t s<sup>o</sup>c c<sup>c</sup>n thi<sup>o</sup>t nh<sup>o</sup>m ð- a ra c<sup>c</sup> bi<sup>o</sup>n ph<sup>p</sup> ph<sup>t</sup> hi<sup>o</sup>n s<sup>i</sup>m, ph<sup>u</sup>ng ch<sup>ê</sup>ng ch<sup>u</sup> ð<sup>é</sup>ng v<sup>u</sup> c<sup>a</sup> hi<sup>o</sup>u qu<sup>u</sup> d<sup>ê</sup>ch b<sup>o</sup>nh do virus Hantaan g<sup>o</sup>y ra ẽ Vi<sup>o</sup>t Nam.

**- T<sup>x</sup>nh h<sup>x</sup>nh nghi<sup>n</sup> c<sup>o</sup>u c<sup>n</sup>a ch<sup>u</sup> nhi<sup>o</sup>m S<sup>o</sup> t<sup>u</sup>i trong l<sup>u</sup>nh v<sup>u</sup>c n<sup>u</sup>y v<sup>u</sup> nh<sup>o</sup>ng v<sup>ê</sup>n ð<sup>o</sup> m<sup>i</sup>i ð<sup>ê</sup>t ra nghi<sup>n</sup> c<sup>o</sup>u.**

Do c<sup>a</sup> qu<sup>u</sup>, nhi<sup>o</sup>u b<sup>o</sup>nh ph<sup>ê</sup>m trong v<sup>o</sup> d<sup>ê</sup>ch SD/ SXHD x<sup>o</sup>y ra v<sup>u</sup>o nh<sup>o</sup>ng n<sup>o</sup>m g<sup>c</sup>n ð<sup>o</sup>y, ch<sup>o</sup>ng t<sup>o</sup>i ð<sup>o</sup> ti<sup>o</sup>n h<sup>u</sup>nh thu th<sup>ê</sup>p v<sup>u</sup> l<sup>u</sup>m ph<sup>u</sup>n ơng huy<sup>o</sup>t thanh th<sup>x</sup> ð<sup>o</sup>:

"B-íc ð<sup>o</sup>u ph<sup>t</sup> hi<sup>o</sup>n nhi<sup>o</sup>m Hantaan virus t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh ð<sup>o</sup>ng b<sup>o</sup>ng mi<sup>o</sup>n B<sup>o</sup>c Vi<sup>o</sup>t Nam 1998- 1999".

C<sup>o</sup>ng v<sup>i</sup>i s<sup>u</sup>

"Gi<sup>o</sup>m s<sup>t</sup> s<sup>u</sup> l- u h<sup>u</sup>nh c<sup>c</sup> type virus Dengue t<sup>i</sup>i Vi<sup>o</sup>t Nam, giai ð<sup>o</sup> 1987- 2001; T<sup>i</sup>p ch<sup>y</sup> Y h<sup>o</sup>c Dù ph<sup>u</sup>ng; 2002: 21- 26".

Ch<sup>o</sup>ng t<sup>o</sup>i c<sup>o</sup>n

"Kh<sup>o</sup> s<sup>t</sup> b-íc ð<sup>o</sup>u v<sup>o</sup> nhi<sup>o</sup>m Virus Hantaan t<sup>i</sup>i m<sup>ê</sup>t s<sup>ê</sup> t<sup>o</sup>nh ð<sup>o</sup>ng b<sup>o</sup>ng B<sup>o</sup>c b<sup>é</sup>. T<sup>i</sup>p ch<sup>y</sup> Y h<sup>o</sup>c Dù ph<sup>u</sup>ng, 2000: 34- 38".

Ti<sup>o</sup>p theo "B-íc ð<sup>o</sup>u nghi<sup>n</sup> c<sup>o</sup>u huy<sup>o</sup>t thanh h<sup>o</sup>c Hantaan virus tr<sup>n</sup> chu<sup>ê</sup>t t<sup>i</sup>i hai t<sup>o</sup>nh H<sup>o</sup>u B<sup>i</sup>nh v<sup>u</sup> L<sup>u</sup>o Cai trong n<sup>o</sup>m 2002; T<sup>i</sup>p ch<sup>y</sup> Y h<sup>o</sup>c th<sup>u</sup>c h<sup>u</sup>nh; 2003: 17- 19".

V<sup>u</sup>: "Nghi<sup>n</sup> c<sup>o</sup>u huy<sup>o</sup>t thanh h<sup>o</sup>c Virus Hantaan tr<sup>n</sup> chu<sup>ê</sup>t t<sup>i</sup>i t<sup>o</sup>nh H<sup>u</sup> Nam trong n<sup>o</sup>m 2002; T<sup>i</sup>p ch<sup>y</sup> Y h<sup>o</sup>c Dù ph<sup>u</sup>ng; 2003: 38- 41".

Vi<sup>o</sup>c l- u h<sup>u</sup>nh virus Hantaan t<sup>i</sup>i Vi<sup>o</sup>t Nam l<sup>u</sup> ð<sup>i</sup>u ch<sup>o</sup>c ch<sup>u</sup>n, nh- ng type virus Hantaan n<sup>u</sup>o g<sup>o</sup>y b<sup>o</sup>nh t<sup>i</sup>i Vi<sup>o</sup>t Nam l<sup>u</sup> ð<sup>i</sup>u h<sup>o</sup>t s<sup>o</sup>c c<sup>c</sup>n thi<sup>o</sup>t...S<sup>o</sup> t<sup>o</sup> ð<sup>o</sup> ñ<sup>u</sup>ng<sup>u</sup>h Y t<sup>o</sup> Vi<sup>o</sup>t Nam c<sup>a</sup> kh<sup>u</sup> n<sup>o</sup>ng h<sup>o</sup>ch ð<sup>o</sup>nh c<sup>c</sup> bi<sup>o</sup>n ph<sup>p</sup> ph<sup>u</sup>ng v<sup>u</sup> ch<sup>ê</sup>ng b<sup>o</sup>nh n<sup>u</sup>y m<sup>ê</sup>t c<sup>c</sup>h ch<sup>u</sup> ð<sup>é</sup>ng v<sup>u</sup> hi<sup>o</sup>u qu<sup>u</sup>....C<sup>o</sup>ng ch<sup>y</sup>nh vi th<sup>o</sup>, ch<sup>o</sup>ng t<sup>o</sup>i m<sup>u</sup>en:

a. Nghi<sup>n</sup> c<sup>o</sup>u c<sup>ê</sup>u tr<sup>o</sup>c gien c<sup>n</sup>a virus Hantaan g<sup>o</sup>y n<sup>n</sup> b<sup>o</sup>nh

sét xuất huyết do virus Hantaan tại khu vực công Hải Phòng  
- Ph. t. hi. n. kh. n. g. th. o. kh. n. g. virus Hantaan b. n. g. c. c. k. u. thu. t.:  
Enzim: ELISA, IF; Si. n. di: Western Blot; RT-PCR tr. n. c. n. g.  
nh. n. c. n. g. v. u. qu. c. n. th. o. chu. t. (x. c. h. o. a. virus Hantaan)

**b. Si. n. tra. d. p. ch. t. o. virus Hantaan**

S. u. kh. c. bi. e. t. gi. a. b. o. n. h. SD/ SXHD và sét xuất huyết do virus Hantaan

X. e. y. d. u. n. g. q. u. i. tr. x. n. h. ch. e. n. o. o. n. sét do virus Hantaan g. o. y. n. a. n.

E. C. c. b. o. n. h. sét cao do virus m. u. kh. n. g. r. a. n. g. u. y. a. n. nh. n.  
Nhi. e. u. b. o. n. h. kh. n. g. r. a. n. g. u. y. a. n. nh. n. ho. a. c. o. l. a. n. g. v. u. n. a. y. l. i. t. r. e. i.  
d. i. y: Chikungunya, West Nile, Nepa...

**Li. e. t. k. a. danh. m. o. c. c. c. n. g. tr. x. n. h. nghi. a. n. c. o. u. c. a. li. a. n. quan:**

**C. n. g. tr. x. n. h. c. n. a. c. c. t. c. gi. a. trong. n. i. c.**

1. Bui gi. n. g. vi. sinh. Y. h. a. c. Nh. u. xu. t. b. i. n. Y. h. a. c. 1993.
2. Bach Thi, Q.N., Truong Uyen, N., Truong Thua, T., Le Thi, M.Q., Nguyen Thi, H.H. and Dinh Duy, K.: Cloning and expression of the gene coding for preM and envelope protein of dengue virus typ 2. ACCESSION AJ574886. (27-JUN-2003).
3. Tr. b. n. h. Qu. y. B. n., Nguy. n. H. i. B. n. g., Tr. n. g. Uy. a. n. Ninh, L. a. Th. p. Qu. n. h. Mai, Nguy. n. Th. p. H. a. n. h. H. i. n. h., S. i. n. h. Duy. Kh. n. g. 2003. T. c. h. d. s. n. g. v. u. bi. e. u. hi. e. n. v. i. n. g. gen. m. h. o. protein preM v. u. protein v. a. c. n. a. vir. u. t. dengue typ 4. T. i. p. ch. y. C. n. g. ngh. o. Sinh. h. a. c. 1:33-38.
4. Dinh, D.K., Le, T.H., Nong, V.H., Phan, V.C., Nguyen, B.N., Nguyen, D.T. and Le, T.B. Molecular analysis of the avian influenza virus H5N1 isolated in poultry in Vietnam during the early 2004 outbreaks: Influenza A virus (A/chicken/Vietnam/HD1/2004(H5N1)) hemagglutinin. ACCESSION AY574187. (16-MAR-2004).
5. Duong Hong, Q., Truong Uyen, N., Truong Thua, T., Le Thi, M.Q., Nguyen Thi, H.H. and Dinh Duy, K.: Cloning and Expression of the genes coding for PreM and Envelope proteins of Dengue virus type 1. ACCESSION AJ574760. (25-JUN-2003).
6. S. e. c. quang. H. u. Nghi. a. n. c. o. u. sét xu. t. h. u. y. e. t. v. o. m. a. t. vir. u. t. h. a. c. trong. 3. n. a. m. 1978-1980 t. i. i. m. e. t. v. u. i. o. p. a. ph. n. g. mi. e. n. Nam. Vi. e. t. nam. Y. h. a. c. Vi. e. t. nam, 1982, 25-26.
7. L. a. th. p. Qu. n. h. Mai, Tr. n. g. uy. a. n. Ninh, Nguy. n. th. p. Th. u. n. h. S. o. s. n. h. 2. th. o. nghi. e. m. MAC-ELISA v. u. DB Dengue Blot trong. ch. e. n. o. o. n. nhanh. v. u. s. i. m. b. o. n. h. sét xu. t. h. u. y. e. t. Dengue. T. i. p. ch. y. Vi. sinh. v. e. t. h. a. c. v. u. C. n. g. ngh. o. sinh. h. a. c., 1995, 184.
8. Ho. u. n. g. Thu. u. Nguy. a. n., Ho. u. n. g. Minh. Tuy. e. t., Tr. n. g. Uy. a. n. Ninh, L. a. v. i. n. Si. e. t., L. a. Qu. n. h. Mai, Nguy. n. th. p. Th. u. n. h. S. i. n. xu. t. kh. n. g. th. o. o. n. clon. kh. n. g. vir. u. t. Dengue typ 1, 2, 3, 4 v. u. o. n. g. d. o. n. g. trong. ch. e. n. o. o. n. nhanh. sét Dengue. T. i. p. ch. y. V. o. sinh. ph. s. n. g. d. p. ch., t. e. p. 4, s. e. 3, 1994, 106-107.
9. Nguyen Tien, M., Truong Uyen, N., Truong Thua, T., Le Thi, M.Q., Nguyen Thi, H.H. and Dinh Duy, K. Cloning and expression of the genes encoding PreM and Envelope proteins of Dengue virus type 3. ACCESSION. (13-MAY-2003).
10. Tr. n. g. Uy. a. n. Ninh, Hu. n. h. Ph. n. g. Li. a. n. Gi. m. s. t. h. u. y. e. t. thanh. h. a. c. b. o. n. h. sét xu. t. h. u. y. e. t. Dengue trong. vi. e. c. s. o. d. o. n. g.

mụn Olyset. Típ chý VÔ sinh phâng dâch, tãp 5, sè 4,95,66-70.

11. Tr-ång Uy-ân Ninh, L-â Qu-ân Mai, Nguy-ôn th-â Th-ân, Tr-ång th-â Th-âng. VÔ kh-âng nguy-ân Dengue s-ân xu-ât tr-ân t-õ bu-ô mu-çi A. Albopictus d-âng C6/36. Típ chý VÔ sinh phâng dâch, tãp 8, sè 2, 1998,132-133.
12. Tr-ång Uy-ân Ninh. Nh-ên x-đt v-ò s-ù ph-ôn b-ê c,c typ virut Dengue ở mi-ôn B-âc Vi-êt nam 1986-1995. Típ chý VÔ sinh phâng dâch, tãp 5, sè 4,1995,51-55.
13. Trinh Quy,B., Truong Uyen,N., Truong Thua,T., Le Thi,M.Q., Nguyen Thi,H.H. and Dinh Duy,K.: Cloning and expression of the genes encoding PreM and Envelope proteins of of Dengue virus type 4. ACCESSION AJ563356. (13-MAY-2003).

**C-âng tr-xnh c-âa c,c t,c gi-â n-íc ngo-âi**

1. Acute respiratory syndrome in China - update 3: disease outbreak reported. Geneva: World Health Organization, February 2003.
2. Balotta,C., Corvasce,S., Violin,M., Galli,M., Moroni,M., Vigevani,G.M., Ruan,Y.J. and Salemi,M. SARS coronavirus AS, complete genome. ACCESSION AY427439. 21-OCT-2003.
3. Bhamarapravati N, Sutee Y. Live attenuated tetravalent dengue vaccine. Vaccine 2000 May 26;18 Suppl 2:44-7.
4. Bisht H, Chugh DA, Swaminathan S, Khanna N. Expression and purification of Dengue virus type 2 envelope protein as a fusion with hepatitis B surface antigen in *Pichia pastoris*. Protein. Expr. Purif. 2001. Vol. 23:84-96.
5. Bright RA, Ross TM, Subbarao K, Robinson HL, Katz JM. Impact of glycosylation on the immunogenicity of a DNA-based influenza H5 HA vaccine. Virology. 2003 Apr 10;308(2):270-8.
6. Clark, D.H, and J.Casls. *Techniques for hemagglutination and hemagglutination inhibition with arthropod-born viruses*. J Trop. Med. Hyg,1958, Vol 7, 561-577.
7. De Paula SO, de Melo Lima C, Torres MP, Pereira MR, Lopes da Fonseca BA. One-Step RT-PCR protocols improve the rate of dengue diagnosis compared to Two-Step RT-PCR approaches. J Clin Virol. 2004, 30(4):297-301.
8. Deubel, V. *The contribution of molecular techniques to the diagnosis of dengue infection*. CAB International, London, United Kingdom, 1997, 335-366.
9. Deubel, V., and V. Pierre. *Molecular techniques for rapid and more sensitive detection and diagnosis of flaviviruses*. Intercept, Andover, United Kingdom, 1994, 227-237.
10. Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J., Guan, Y. J., Butt, K. M., Wong, K. L., Chan, K. W., Lim, W., Shortridge, K. F., Yuen, K. Y., Peiris, J. S. M., Poon, L. L. M. (2003). Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China. *Science* 302: 276-278
11. Henschel, E. A., J.M.McCown,M.C.Sequin, M.K. Gentry, and W.E. Brandt. *Rapid identification of dengue virus*



- isolates by using monoclonal antibodies in an indirect immunofluorescence assay. *Am. Trop. Med. Hyg*, 1983, vol 32,164-169.
12. Hermida L, Rodriguez R, Lazo L, Lopez C, Marquez G, Paez R, Suarez C, Espinosa R, Garcia J, Guzman G, Guillen G. A recombinant envelope protein from Dengue virus purified by IMAC is bioequivalent with its immune-affinity chromatography purified counterpart. *J. Biotechnol.* 2002. Vol. 94:213-6.
  13. Houngh HS, Norwood D, Ludwig GV, Sun W, Lin M, Vaughn DW. Development and evaluation of an efficient 3'-noncoding region based SARS coronavirus (SARS-CoV) RT-PCR assay for detection of SARS-CoV infections. *J Virol Methods.* 2004 Sep;120:33-40.
  14. Kawano, H., V.Rostapshow, L.Rosen, and C.J.lai. Genetic determinant of dengue typ-4 virus neurovirulence for mice. *J. Vil*, 1993, vol 67, 6567-6575.
  15. Kliks.S.C, A. Nisalak, W.E.Brandt, L.Wahl, D.S.Bruke. Antibody- Dependent enhancement of dengue virus growth in human monocyte as a risk factor for dengue hemorrhagic fever. *Am. Trop. Med.Hyg*, 1989,vol 40, 444-451.
  16. Kuno, G., D. J. Gubler, and A. Oliver. Use of original antigenic sin theory to determine the serotypes of previous dengue. *Trans.R.Trop. Med. Hyg*,1993, vol 87,103-105.
  17. Kuno, G., D. J. Gubler, and I. Gomez. An ELISA procedure for the diagnosis of dengue infections. *J.Virol.Methods*, 1991, vol 33, 101-103.
  18. Lam, S. K., M.Y.Fong, E. Chungue, S.Doraisingham, A.Igarashi, M.A.Khin, Z. T. Kyaw, A. Nisalak, C. Roche, D.W.Vaughn, and V.Vordam. Multicentre evalution of dengue IgM dot enzyme immunoassay. *Clin. Diagn. Viol*, 1996, vol 7,93-98.
  19. Lanciotti, R. S., C.H. Calisher, D.J.Gubler, and D.W.Tren. Molecular evolution and epidemiology of dengue-3 viruses. *J.Gen.Viol*, 1994,vol 75,65-75.
  20. Lau LT, Fung YW, Wong FP, Lin SS, Wang CR, Li HL, Dillon N, Collins RA, Tam JS, Chan PK, Wang CG, Yu AC. A real-time PCR for SARS-coronavirus incorporating target gene pre-amplification. *Biochem Biophys Res Commun.* 2003 Dec 26;312(4):1290-6.
  21. Le van Diet, Truong .U. N., Huynh.P.L., Hoang.T.N.,K.Morita, and A.Igarashi. Application of RT-PCR for serotype determination of Dengue virus strain isolated in North Vietnam, 1991-1993. *Trop. Med*, 1994, vol 36, 111-115.
  22. Lemmer K, Donoso Mantke O, Bae HG, Groen J, Drosten C, Niedrig M. External quality control assessment in PCR diagnostics of dengue virus infections. *J Clin Virol.* 2004, 30(4):291-6.
  23. Maneekarn N, Morita K, Tanaka M, Igarashi A, Usawattanakul W, Sirisanthana V, Innis BL, Sittisombut N, Nisalak A, Nimmanitya S. Applications of polymerase chain reaction for identification of dengue viruses isolated from patient sera. *Microbiol Immunol.* 1993, 37(1):41-7.
  24. Morita, K., M.Takana, and A.Igarashi. Rapid

- identification of dengue virus serotype by using PCR. J.Clin.Micobiol, 1991, 2107-2110.
25. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:263-73.
  26. Poddar SK. Influenza virus types and subtypes detection by single step single tube multiplex reverse transcription-polymerase chain reaction (RT-PCR) and agarose gel electrophoresis. : J Virol Methods. 2002 Jan;99(1-2):63-70.
  27. Poon LL, Chan KH, Wong OK, Yam WC, Yuen KY, Guan Y, Lo YM, Peiris JS. Early diagnosis of SARS coronavirus infection by real time RT-PCR. J Clin Virol. 2003 Dec;28(3):233-8
  28. Poon LL, Wong BW, Chan KH, Leung CS, Yuen KY, Guan Y, Peiris JS. A one step quantitative RT-PCR for detection of SARS coronavirus with an internal control for PCR inhibitors. J Clin Virol. 2004 Jul;30(3): 214-7.
  29. Ruan, Y., Wei, C.L., Ling, A.E., Vega, V.B., Thoreau, H., Se Thoe, S.Y., Chia, J.-M., Ng, P., Chiu, K.P., Lim, L., Zhang, T., Chan, K.P., Oon, L.E.L., Ng, M.L., Leo, S.Y., Ng, L.F.P., Ren, E.C., Stanton, L.W., Long, P.M. and Liu, E.T. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 361 (9371), 1779-1785 (2003).
  30. Ruechusatsawat, K., K. Morita, M. Tanakka. Daily observation of antibody levels among dengue patients detected by ELISA. Jpn. J. Trop. Med. Hyg, 1994, vol 22, 9-12.
  31. Russell, P. K., and A. Nisalak. Plaque reduction test for dengue virus neutralizing antibodies. J. Immunol, 1987, vol 99, 285-290.
  32. Samuel R, Jesudass ES, Myers RM. Complement fixation tests with cell culture fluid as antigen in comparison with cell culture neutralization tests in the identification of freshly isolated dengue virus: a pilot study. Indian. J. Med. Res. 1973. Vol. 61:1725-9.

**11 C, ch tiếp cỄn, ph-~~ng~~ ph, p nghi^n cou, ku thuệt si so đong**

TẾp tho thuc hion oo tui (bao gam nhiou chuyan gia trong c, c lunh vuc nghi^n cou nuy) o. tiop cỄn ocy oñ c, c thong tin cong nh- c, c vut liou tui con thiot nh- virut Dengue c, c typ I, II, III, IV; To buo *Aedes albopictus* đung C6/36; C, c ho, chut; C, c lo'i conjugate; C, c gi, tho; Virus Corona goy bonh SARS, virus com H5N1... oo houn thunh yau cu c, c nui dung nghi^n cou tr-ic tian c, c muo bonh phum nuy o-ic sung luc u c, c phung thuy nghiom chuun thoc ca Vion Vo sinh Đuch to Trung --ng...Tui oiou kion mu ca tho đung c, c test chun ođun nhanh hay ku thuệt IgM ELISA, IFA, PRNT, RT- PCR...Sau khi laai tro c, c bonh o. biot nh- virus Com, Virus Dengue, Virus Hantaan, Virus Viam no Nhut buo B, Virus Viam gan, sut rut...Bonh phum si o-ic c, c nhu khoa hac ti Vion VSDTT, si hac Y to Cong cung, APITMID tiop toc nghi^n cou oo txm cun nguyan sou xa...

C.c mẾu kh«ng r« nguyªn nh«n ®-íc g«i Ồn APITMID, Honolulu, Hoa kú Ồ c.c nhự khoa h«c ViÕt Nam vự Hoa Kú nghiªn cøu thªm. Tªi Ồy sũ dĩng c.c kũ thuỆt hiÕn Ồi nh-: Nhuэм m« h«a miÕn dỈch, chÝp ỒiÕn tũ cho Gen vự Protein, sinh h«c ph«n tũ vự ph«n lỄp bỖnh phỄm...ph«n tÝch vự lỄp cỖy phª hỖ...

## 12 Néi dung nghiªn cøu

Dù n nựy sũ ®-íc tiÕn hựnh qua hai giai Ồn.

Giai Ồn I bao gảm mỖc tiªu mét vự hai.

Giai Ồn II sũ bao gảm mỖc tiªu ba vự bèn.

Giai Ồn II sũ chỖ ®-íc thục hiÕn sau khi giai Ồn I ®. hựp thựnh tèt Ồp.

Trong giai Ồn I, hỖ thềng gi,m s,t c.c hếi chøng sũ ®-íc thựnh lỄp Ồ theo d«i c.c bỖnh sèt do nhiêm virus ể miÕn B½c ViÕt Nam. SỈa ỒiÕm gi,m s,t gảm c«: BỖnh viÕn B½ch Mai ể Hự Néi, BỖnh viÕn tỖnh L½ng S-n, BỖnh viÕn ViÕt TiỖp ể thựnh phè H½i Ph½ng, BỖnh viÕn tỖnh H½a B½nh vự BỖnh viÕn tỖnh Cao B½ng. Ng½ai ra cỈn c« cª mét sè Tr½m y tũ vự ph½ng kh,m t- sũ ®-c ch«n lựm ỒiÕm gi,m s,t trong thựnh thỈ vự v½ng n«ng th«n. C.c mẾu huyỐt thanh sũ ®-íc lỄy tũ c.c bỖnh nh«n Ồn kh,m vự Ồang ®-íc ỒiÕu trỈ vớ bỖnh cªnh sèt cao kh«ng r« nguyªn nh«n. Nh½ng ng-ếi bỖnh vớ triÕu chøng sèt sau Ồy sũ ®-íc Ồ«c biÕt quan tỖm:

- 1.Cªn nguyªn do virus
- 2.Viªm ®-ềng h« hỄp cỄp tÝnh
- 3.Sèt t-«ng tù nh- bỖnh Dengue
- 4.Sèt Ồi kỈm vớ xuỆt huyỐt
- 5.Sèt Ồi kỈm vớ c.c bỖnh vỖ n.Ỗ
- 6.Sèt kh«ng r« nguyªn nh«n vự dĩn Ồn tũ vong

ChỖ thu thỄp huyỐt thanh ể c.c bỖnh nh«n vớ cªn nguyªn do virus, Viªm ®-ềng h« hỄp cỄp tÝnh vự Sèt Dengue. Sè l-½ng mẾu dỈch phỖ thuéc vự khª n½ng xỖ lý c«a ph½ng thÝ nghiõm tªi ViÕn VỖ sinh DỈch tỖ Trung -«ng vự tuú thuéc bỖnh dỈch Ồang xªy ra hay kh«ng. TỆt cª bỖnh nh«n bỈ viªm ph«i, sèt xuỆt huyỐt, bỖnh vỖ n.Ỗ hay Sèt kh«ng r« nguyªn nh«n dĩn Ồn tũ vong tªi c.c tr½m gi,m s,t sũ ®-íc lỄy mẾu. HỖ thềng gi,m s,t nựy sũ ®-íc tiỖp tỖc thục hiÕn qu«nh n½m, trỖ khi c« bỖnh dỈch xªy ra. Trong tr-ềng híp dỈch bỖnh xªy ra, tiªu chuỄn gi,m s,t sũ ®-íc ỒiÕu chỖnh.

Nh½ng mẾu bỖnh phỄm nựy (m,u, n-íc b«t, dỈch n.Ỗ tuú hay tũ bựo tuú theo triÕu chøng bỖnh) sũ ®-íc thu thỄp tũ bỖnh nh«n vự ®-íc bỈo qu«n trong tũ ®«ng l½nh hay trong th½ng chỖa dung dỈch Nitrogen. MẾu m,u sũ ®-íc thu thỄp tũ tỆt cª bỖnh nh«n vự cỆt vự tube th½y t½nh c« dung tÝch 10 ml, huyỐt thanh sũ ®-íc t, ch vự cỆt vự hai cryo tube, bỈo qu«n trong l½nh sỖu. Khi c« c- hếi, c« thỖ thu thỄp n-íc n.Ỗ tuú ể bỖnh nh«n nghi viªm mựng n.Ỗ. Dung dỈch ngo,y h«ng ể b«nh nh«n viªm ®-ềng h« hỄp cỖng ®-íc thu thỄp vự bỈo qu«n Ồóng tiªu chuỄn. NỖu c« bỖnh nh«n tũ vong nghi ngê, cỈn m« x,c hay lựm sinh thiỐt. Theo Ồpnh kú, c.c mẾu bỖnh phỄm sũ ®-íc g«i tũ ViÕn VỖ sinh DỈch tỖ Trung -«ng

t<sup>1</sup>i Hụ Néi qua @-êng b-u @iôn hoặ c,c ph-<sup>ng</sup> tiôn v<sup>ên</sup> chuyôn. Bôn<sup>h</sup> ph<sup>ê</sup>m s<sup>ĩ</sup> @-íc @ă<sup>ng</sup> v<sup>u</sup> di chuyôn trong th<sup>ĩ</sup>ng b<sup>ĩ</sup>o qu<sup>ĩ</sup>n l<sup>ĩ</sup>nh v<sup>ĩ</sup>i c,c qui @<sup>ĩ</sup>nh nghi<sup>ĩ</sup>m ng<sup>ĩ</sup>t v<sup>o</sup> y<sup>ĩ</sup>u c<sup>ũ</sup>u k<sup>ĩ</sup> thu<sup>ĩ</sup>t.

Tr-íc ti<sup>ĩ</sup>n, c,c m<sup>ĩ</sup>u bôn<sup>h</sup> ph<sup>ê</sup>m nuy @-íc s<sup>ũ</sup>ng lăc ẽ c,c Ph<sup>ĩ</sup>ng th<sup>ĩ</sup>y nghi<sup>ĩ</sup>m chu<sup>ĩ</sup>n th<sup>o</sup>c c<sup>ĩ</sup>a Vi<sup>ĩ</sup>ôn NIHE. T<sup>ĩ</sup>y v<sup>u</sup> @i<sup>o</sup>u ki<sup>o</sup>n m<sup>ũ</sup> c<sup>ĩ</sup>a th<sup>o</sup> đ<sup>ĩ</sup>ng c,c test ch<sup>ĩ</sup>n @o,n nh<sup>ĩ</sup>nh nh- IgM ELISA. IFA, PRNT, RT-PCR...Sau khi lăai tr<sup>o</sup>c c,c bôn<sup>h</sup> @- bi<sup>o</sup>t nh- virus Dengue, virus c<sup>o</sup>m, virus vi<sup>ĩ</sup>m n<sup>o</sup>, s<sup>ĩ</sup>t r<sup>ĩ</sup>đt...Bôn<sup>h</sup> ph<sup>ê</sup>m s<sup>ĩ</sup> @-íc c,c nh<sup>ũ</sup> khoa hăc t<sup>ĩ</sup>i Vi<sup>ĩ</sup>ôn V<sup>ĩ</sup> sinh đ<sup>ĩ</sup>ch t<sup>o</sup> Tr<sup>ũ</sup>ng -<sup>ũ</sup>ng v<sup>u</sup> APITMID ti<sup>o</sup>p t<sup>o</sup>c nghi<sup>ĩ</sup>n c<sup>o</sup>u @<sup>o</sup> t<sup>ĩ</sup>xm c<sup>ĩ</sup>n nguy<sup>ĩ</sup>n...

T<sup>ĩ</sup>t c<sup>ũ</sup> c,c m<sup>ĩ</sup>u bôn<sup>h</sup> ph<sup>ê</sup>m kh<sup>ĩ</sup>ng r<sup>ĩ</sup>a nguy<sup>ĩ</sup>n nh<sup>o</sup>n s<sup>ĩ</sup> @-íc g<sup>o</sup>i t<sup>ĩ</sup>i Vi<sup>ĩ</sup>ôn APITMID t<sup>ĩ</sup>i th<sup>ũ</sup>nh ph<sup>ĩ</sup> Honolulu @<sup>o</sup> c,c nh<sup>ũ</sup> khoa hăc Vi<sup>ĩ</sup>ôt Nam v<sup>u</sup> Hoa K<sup>ĩ</sup> nghi<sup>ĩ</sup>n c<sup>o</sup>u th<sup>ĩ</sup>m. T<sup>ĩ</sup>i @<sup>o</sup>y s<sup>ĩ</sup> đ<sup>ĩ</sup>ng c,c k<sup>ĩ</sup> thu<sup>ĩ</sup>t chu<sup>ĩ</sup>n @o,n hi<sup>o</sup>n @<sup>ĩ</sup>i nh- nhu<sup>ĩ</sup>m m<sup>ũ</sup> ho, mi<sup>o</sup>n đ<sup>ĩ</sup>ch, ch<sup>ĩ</sup>y p<sup>ĩ</sup> @i<sup>o</sup>n t<sup>o</sup> cho Gen v<sup>u</sup> Protein, sinh hăc ph<sup>ĩ</sup>n t<sup>o</sup> v<sup>u</sup> ph<sup>ĩ</sup>n l<sup>ĩ</sup>p tr<sup>ĩ</sup>n m<sup>ũ</sup>i bôn<sup>h</sup> ph<sup>ê</sup>m. Khi ph<sup>ĩ</sup>t hi<sup>o</sup>n ra ch<sup>ĩ</sup>ng gi<sup>ĩ</sup>ng g<sup>o</sup>y bôn<sup>h</sup> l<sup>ĩ</sup>, Gen s<sup>ĩ</sup> @-íc t<sup>ĩ</sup>ch ra t<sup>o</sup> c,c m<sup>ĩ</sup>u bôn<sup>h</sup> ph<sup>ê</sup>m v<sup>u</sup> @-íc ph<sup>ĩ</sup>n t<sup>ĩ</sup>ch v<sup>u</sup> l<sup>ĩ</sup>p c<sup>o</sup>y ph<sup>ĩ</sup> h<sup>o</sup> t<sup>ĩ</sup>i APITMID. N<sup>o</sup>u l<sup>ũ</sup> t<sup>ĩ</sup>c nh<sup>o</sup>n ch-<sup>ĩ</sup>a t<sup>o</sup>ng bi<sup>o</sup>t @<sup>o</sup>n, nă s<sup>ĩ</sup> @-íc @<sup>ĩ</sup>t t<sup>ĩ</sup>n v<sup>u</sup> c<sup>ũ</sup>ng tr<sup>ĩ</sup>xnh nghi<sup>ĩ</sup>n c<sup>o</sup>u s<sup>ĩ</sup> @-íc c<sup>ũ</sup>ng b<sup>ĩ</sup> tr<sup>ĩ</sup>n t<sup>ĩ</sup>p tr<sup>ĩ</sup>y khoa hăc v<sup>u</sup> c,c nh<sup>ũ</sup> nghi<sup>ĩ</sup>n c<sup>o</sup>u khoa hăc Vi<sup>ĩ</sup>ôt Nam @ă<sup>ng</sup> vai tr<sup>ĩ</sup> quan tră<sup>ĩ</sup>ng.

Nh<sup>ũ</sup>ng t<sup>ĩ</sup>c nh<sup>o</sup>n g<sup>o</sup>y bôn<sup>h</sup> m<sup>ĩ</sup>i s<sup>ĩ</sup> l<sup>ũ</sup> tră<sup>ĩ</sup>ng t<sup>o</sup>m trong Ch-<sup>ũ</sup>ng tr<sup>ĩ</sup>xnh nghi<sup>ĩ</sup>n c<sup>o</sup>u. Sau khi th<sup>ũ</sup>ng qua H<sup>ĩ</sup>i @ă<sup>ng</sup> §<sup>ĩ</sup>o @<sup>o</sup>c Y hăc, §HYTCC, vi<sup>ĩ</sup>ôn VSDTT, v<sup>u</sup> vi<sup>ĩ</sup>ôn APITMID s<sup>ĩ</sup> b<sup>ĩ</sup>t @<sup>ũ</sup>u nghi<sup>ĩ</sup>n c<sup>o</sup>u t<sup>ĩ</sup>i hi<sup>o</sup>n tr-êng @<sup>o</sup> t<sup>ĩ</sup>xm ra ẽ ch<sup>o</sup>a trong thi<sup>ĩ</sup>n nh<sup>ĩ</sup>n, ph-<sup>ũ</sup>ng th<sup>o</sup>c truy<sup>o</sup>n nhi<sup>o</sup>m, t<sup>ĩ</sup>m quan tră<sup>ĩ</sup>ng @<sup>ĩ</sup>i v<sup>ĩ</sup>i s<sup>o</sup>c kho<sup>ĩ</sup> v<sup>u</sup> t<sup>ĩ</sup>xm hi<sup>o</sup>u v<sup>o</sup> sinh th<sup>ĩ</sup>i c<sup>ĩ</sup>a m<sup>ũ</sup>m bôn<sup>h</sup>. N<sup>o</sup>u ph<sup>ĩ</sup>t hi<sup>o</sup>n c,c bôn<sup>h</sup> m<sup>ĩ</sup>i s<sup>ĩ</sup> @-íc c<sup>ũ</sup>ng b<sup>o</sup> nh- l<sup>ũ</sup> c<sup>ĩ</sup>n nguy<sup>ĩ</sup>n ti<sup>o</sup>m t<sup>ũ</sup>ng g<sup>o</sup>y đ<sup>ĩ</sup>ch bôn<sup>h</sup> v<sup>u</sup> s<sup>ĩ</sup> đ<sup>ũ</sup>a v<sup>u</sup> @ă @<sup>o</sup> s<sup>ĩ</sup>n xu<sup>ĩ</sup>t c,c test ch<sup>ĩ</sup>n @o,n hoặ l<sup>ũ</sup>m c<sup>ĩ</sup> s<sup>ĩ</sup> nghi<sup>ĩ</sup>n c<sup>o</sup>u cho vaccine ph<sup>ĩ</sup>ng bôn<sup>h</sup> nuy.

C,c nh<sup>ũ</sup> khoa hăc Vi<sup>ĩ</sup>ôt Nam v<sup>u</sup> Hoa K<sup>ĩ</sup> c<sup>ĩ</sup>a vi<sup>ĩ</sup>ôn APITMID s<sup>ĩ</sup> ph<sup>ĩ</sup>n t<sup>ĩ</sup>ch v<sup>u</sup> l<sup>ĩ</sup>p c<sup>o</sup>y ph<sup>ĩ</sup> h<sup>o</sup> t<sup>o</sup> gen c<sup>ĩ</sup>a c,c m<sup>ũ</sup>m bôn<sup>h</sup> m<sup>ĩ</sup>i nuy. T<sup>ĩ</sup>t c<sup>ũ</sup> th<sup>ũ</sup>ng tin s<sup>ĩ</sup> @-íc chia s<sup>ĩ</sup> v<sup>ĩ</sup>i c,c c<sup>ĩ</sup> quan nh<sup>ũ</sup> n-íc Vi<sup>ĩ</sup>ôt Nam c<sup>o</sup>ng nh- v<sup>ĩ</sup>i c,c c<sup>ĩ</sup> quan Y t<sup>o</sup> Hoa K<sup>ĩ</sup> v<sup>u</sup> Qu<sup>ĩ</sup>c T<sup>o</sup>.

C<sup>ũ</sup>ng vi<sup>ĩ</sup>o c<sup>o</sup> th<sup>o</sup>:

1. Thu th<sup>ĩ</sup>p c,c huy<sup>o</sup>t thanh bôn<sup>h</sup> nh<sup>o</sup>n s<sup>ĩ</sup>t cao kh<sup>ĩ</sup>ng r<sup>ĩ</sup>a nguy<sup>ĩ</sup>n nh<sup>o</sup>n t<sup>ĩ</sup>i Hụ Néi, Cao b<sup>ũ</sup>ng, L<sup>ĩ</sup>ng S<sup>ĩ</sup>n v<sup>u</sup> H<sup>ĩ</sup>i Ph<sup>ĩ</sup>ng.
2. Ti<sup>o</sup>n h<sup>ũ</sup>nh s<sup>ũ</sup>ng lăc c,c m<sup>ĩ</sup>u bôn<sup>h</sup> ph<sup>ê</sup>m b<sup>ũ</sup>ng k<sup>ĩ</sup> thu<sup>ĩ</sup>t: IGM-ELISA, IFA, PRNT, RT-PCR
3. G<sup>o</sup>i c,c m<sup>ĩ</sup>u bôn<sup>h</sup> ph<sup>ê</sup>m @<sup>o</sup>n APITMID, Honolulu, Hoa k<sup>ĩ</sup> @<sup>o</sup> ti<sup>o</sup>p t<sup>o</sup>c ph<sup>ĩ</sup>n t<sup>ĩ</sup>ch b<sup>ũ</sup>ng c,c k<sup>ĩ</sup> thu<sup>ĩ</sup>t cao nh<sup>ũ</sup>m t<sup>ĩ</sup>xm c<sup>ĩ</sup>n nguy<sup>ĩ</sup>n

13 **H<sup>ĩ</sup>p t<sup>ĩ</sup>c qu<sup>ĩ</sup>c t<sup>o</sup>**

T <sup>ĩ</sup> n @ <sup>ĩ</sup> i t <sup>ĩ</sup> c		N <sup>ĩ</sup> i đ <sup>ũ</sup> ng h <sup>ĩ</sup> p t <sup>ĩ</sup> c
<b>S<sup>ĩ</sup> v<sup>u</sup> @ă<sup>ng</sup> h<sup>ĩ</sup>p t<sup>ĩ</sup>c</b>	§ <sup>ĩ</sup> i hăc Y- § <sup>ĩ</sup> i hăc hăc Tă <sup>ĩ</sup> ng h <sup>ĩ</sup> p Hawaii	Nghi <sup>ĩ</sup> n c <sup>o</sup> u v <sup>o</sup> gene c,c type Dengue
		Ch <sup>ĩ</sup> @ <sup>ĩ</sup> ng ph <sup>ĩ</sup> t hi <sup>o</sup> n c,c bôn <sup>h</sup> m <sup>ĩ</sup> i t <sup>ĩ</sup> i Vi <sup>ĩ</sup> ôt

				Nam
14 Tiốn để thực hiện				
TT	C, c néi dung, c«ng viöc thùc hiöu chñ yÖu (C, c m«c ®, nh gi, chñ yÖu)	Sqñ phöem phñi ®¹t	Thêi gian (B§-KT)	Ng-êi, cñ quan thùc hiöu
1	2	3	4	5
1	Thu thËp huyÖt thanh máu	1.000 nếu	01-1	BV. B¹ch Mai, Hø Néi BV. ViÖt TiÖp, H¶I Phñng, BV. L¹ng S-n, Trung t¸m Y tÖ dù phñng t¸nh Cao B»ng
2	Súng lác huyÖt thanh máu			
	IgM-ELISA		04- 12	ViÖn VSDTT
	IFA		05- 12	ViÖn VSDTT
	RT-PCR		08- 12	ViÖn VSDTT
	PRNT		07- 12	ViÖn VSDTT
3	Phön tÝch huyÖt thanh bÖnh nhön sèt cao kh«ng râ nguyªn nhön		10- 12	APITMID
4	ViÖt b, o c, o, viÖt b, o		01/ 2008	NIHE và APITMID

### III. K¸t qu¶ cña ®Ò tµi.

<b>15</b>	<b>D¹ng k¸t qu¶ dù kiõn cña ®Ò tµi</b>		
	<b>I</b>	<b>II</b>	<b>III</b>
	♦ M¸u (model, maket)	♦ Quy tr×nh c«ng nghÖ	♦ S-n ®¸
		☒ p dông qui tr×nh kü thuËt ELISA, IFA, PRNT, RT-PCR ®Ó chÈn ®o, n c, c huyÖt thanh trªn.	
	♦ Sqñ phöem ☒ Thu thËp 1.000 m¸u HT bÖnh nhön	♦ Ph-ng ph,p	♦ B¶ng sè liÖu
	♦ VËt liÖu	♦ Tiªu chuÈn	♦ B, o c, o phön tÝch ☒

♦ Kh, c  
- 01 bụi b, o.  
- Số t' o: 01  
ThS.

**16 Y' u c' u khoa h' c' ài v' i' s' n' ph' m' t' o' ra (d' i' ng k' o' t' qu' I III)**

TT	T' n' s' n' ph' m'	Y' u c' u khoa h' c'	Ch' o' th' y' c'
1	2	3	4
1	B, o c, o ph' o' n' t' y' c' h' C, c b, o c, o v' u' H' e' i' th' o' khoa h' c'	Ph' o' n' t' y' c' h' @, nh gi, nguy' n' nh' o' n' c, c v' o' d' p' ch' s' e' t' cao kh' < ng r' a' nguy' n' nh' o' n'	
2	C, c b' u' i' b, o	Trong n- i' c: 1 Ng' o' u' i' n- i' c: 1	
3	S' u' o' t' o: 01 Th' i' c' s' u'	S' i' m' b' i' o' c' a' t' r' x' nh' @' e' chuy' n' m' < n' trong l' u' nh' v' u' c' < ng ngh' o' gen, c' < ng ngh' o' protein. N' a' m' v' i' ng c, c k' u' thu' e' t' v' o' SHPT, h' a' a' sinh, mi' o' n' d' p' ch' @' o' c' a' th' o' o' ng' d' o' ng c, c k' u' thu' e' t' thu' e' c' l' u' nh' v' u' c' < ng' ngh' o' sinh h' a' c' v' u' o' ph' o' c' v' o' s' n' xu' e' t'.	

**1 Y' u c' u k' u' thu' e' t' , ch' o' t' i' u' ch' e' t' l- i' ng' ài v' i' s' n' ph' m' t' o' ra (d' i' ng k' o' t' qu' I, II)**

TT	T' n' s' n' ph' e' m' v' u' ch' o' t' i' u' ch' e' t' l- i' ng ch' a' y' o' u'	S' n' v' p' @' o'	M' o' c' ch' e' t' l- i' ng			D' u' ki' o' n' s' e' l- i' ng s' n' ph' e' m' t' i' o' ra
			C' c' n' @' i' t'	M' e' u' t- i' ng t' u'		
				Trong n- i' c'	Th' o' gi' i'	
1	2	3	4	5	6	7
	Huy' o' t' thanh b' o' nh' nh' o' n'	m' e' u'	1.000			S' i' m' b' i' o' ch' e' t' l- i' ng ph' o' c' v' o' c, c' x' d' t' nghi' o' m'

**18 Ph- i' ng' th' o' c' chuy' o' n' giao k' o' t' qu' nghi' n' c' o' u**

Qui t' r' x' nh' c' < ng ngh' o' s' n' xu' e' t' kh, ng nguy' n' t, i' t' a' h' i' p' virut Dengue c, c' type I, II, III, IV d' i' ng l' u' m' nguy' n' li' o' u' s' n' xu' e' t' B' e' sinh ph' e' m' ch' e' n' @' o, n' nhanh b' o' nh' SD/ SXHD s' i' @- i' c' chuy' o' n' giao cho c, c' Vi' o' n' khu v' u' c' , c, c' Trung t' o' m' Y' t' o' D' u' ph' i' ng' c, c' t' o' nh/ t' u' nh' trong c' a' n- i' c' @' o' th' u' c' hi' o' n' ph- i' ng' ch' o' m' Y' t' o' D' u' ph' i' ng: Ch' i' @' e' ng' ph' i' ng' ch' e' ng' b' o' nh' SD/ SXHD. Gi' a' m' t' u' l' o' m' a' c' v' u' t' u' l' o' ch' o' t' b' i' o' v' o' s' o' c' kho' i' cho' t' o' n' d' o' n'.

19	<p><b>C, c t, c óéng của kết quả nghiên cứu</b> (ngoại t, c óéng ở n<sup>h</sup>u t<sup>h</sup>i mốc 18 tr<sup>h</sup>an ócy)</p> <ul style="list-style-type: none"> <li>Bài d-ìng, óuo t<sup>h</sup>o c, n bé KH&amp;CN Suo t<sup>h</sup>o 1 ThS. trong l<sup>h</sup>nh v<sup>h</sup>c của óò tui.</li> <li>Sèi v<sup>h</sup>i l<sup>h</sup>nh v<sup>h</sup>c khoa h<sup>h</sup>c cả li<sup>h</sup>n quan:</li> </ul> <p>Ph, t hi<sup>h</sup>on virus m<sup>h</sup>i óó ch<sup>h</sup> óéng ng<sup>h</sup>n ng<sup>h</sup>o c, c vô d<sup>h</sup>ch l<sup>h</sup>n se x<sup>h</sup>y ra cho c<sup>h</sup>ng óang đon c- khu v<sup>h</sup>c ph<sup>h</sup>ya B<sup>h</sup>á Vi<sup>h</sup>ot Nam v<sup>h</sup> c<sup>h</sup> v<sup>h</sup>ng S<sup>h</sup>ng Nam ch<sup>h</sup>u A n<sup>h</sup>i chung</p> <ul style="list-style-type: none"> <li>Sèi v<sup>h</sup>i kinh t<sup>h</sup> - x<sup>h</sup>. héi:</li> </ul> <p>C<sup>h</sup>nh b, o, ng<sup>h</sup>n ch<sup>h</sup>en c, c vô d<sup>h</sup>ch, s<sup>h</sup>n xu<sup>h</sup>t c, c bé kit ch<sup>h</sup>en ó<sup>h</sup>an v<sup>h</sup> cả th<sup>h</sup>ó cả c, c l<sup>h</sup>ai Vacxin m<sup>h</sup>i ph<sup>h</sup>ng b<sup>h</sup>nh cho nh<sup>h</sup>on đon trong t-<sup>h</sup>ng lai</p>
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#### IV. C, c t<sup>h</sup> ch<sup>h</sup>c/c, nh<sup>h</sup>on tham gia th<sup>h</sup>ch hi<sup>h</sup>on óò tui

20	<p><b>Ho<sup>h</sup>t óéng của c, c t<sup>h</sup> ch<sup>h</sup>c phải h<sup>h</sup>p tham gia th<sup>h</sup>ch hi<sup>h</sup>on óò tui</b> (Ghi t<sup>h</sup>et c<sup>h</sup> c, c t<sup>h</sup> ch<sup>h</sup>c phải h<sup>h</sup>p th<sup>h</sup>ch hi<sup>h</sup>on óò tui v<sup>h</sup> ph<sup>h</sup>cn néi dung c<sup>h</sup>ng vi<sup>h</sup>ot tham gia trong óò tui)</p>		
TT	T <sup>h</sup> n t <sup>h</sup> ch <sup>h</sup> c	S <sup>h</sup> pa ch <sup>h</sup>	Ho <sup>h</sup> t óéng/ó <sup>h</sup> ng g <sup>h</sup> p cho óò tui
1	<b>Vi<sup>h</sup>on v<sup>h</sup>ó S<sup>h</sup>nh đ<sup>h</sup>ch t<sup>h</sup>ó Tr<sup>h</sup>ng, -<sup>h</sup>ng, Bé Y T<sup>h</sup>ó</b>	Sè 1 Phè Yersin, Qu <sup>h</sup> en Hai B <sup>h</sup> Tr- <sup>h</sup> ng, H <sup>h</sup> Néi	<ol style="list-style-type: none"> <li>Ph-<sup>h</sup>ng ph, p nu<sup>h</sup>i c<sup>h</sup>ý virus Dengue c, c typ I, II tr<sup>h</sup>n t<sup>h</sup>ó b<sup>h</sup>o Aedes albopictus đ<sup>h</sup>ng C6/36.</li> <li>Ph-<sup>h</sup>ng ph, p nu<sup>h</sup>i c<sup>h</sup>ý virus Dengue c, c typ III, IV tr<sup>h</sup>n t<sup>h</sup>ó b<sup>h</sup>o Aedes albopictus đ<sup>h</sup>ng C6/36.</li> <li>XĐt nghi<sup>h</sup>om h<sup>h</sup>y<sup>h</sup>ot thanh b<sup>h</sup>ng c, c k<sup>h</sup> thu<sup>h</sup>t: - IgM- ELISA - IFA - PRNT - RT- PCR</li> <li>Ph<sup>h</sup>on l<sup>h</sup>ép virus tr<sup>h</sup>n c, c đ<sup>h</sup>ng t<sup>h</sup>ó b<sup>h</sup>o th<sup>h</sup>y<sup>h</sup>ch h<sup>h</sup>p</li> </ol>
2	<b>B<sup>h</sup>nh vi<sup>h</sup>on B<sup>h</sup>ch Mai</b>	H <sup>h</sup> Néi	Thu th <sup>h</sup> ép h <sup>h</sup> y <sup>h</sup> ot thanh b <sup>h</sup> nh nh <sup>h</sup> on
3	<b>B<sup>h</sup>nh vi<sup>h</sup>on Vi<sup>h</sup>ot - Ti<sup>h</sup>óp</b>	H <sup>h</sup> i Ph <sup>h</sup> ng	Thu th <sup>h</sup> ép h <sup>h</sup> y <sup>h</sup> ot thanh b <sup>h</sup> nh nh <sup>h</sup> on
4	<b>BV. T<sup>h</sup>nh L<sup>h</sup>ng S-<sup>h</sup>n</b>	L <sup>h</sup> ng S- <sup>h</sup> n	Thu th <sup>h</sup> ép h <sup>h</sup> y <sup>h</sup> ot thanh b <sup>h</sup> nh nh <sup>h</sup> on
5	<b>TT Y t<sup>h</sup>ó D<sup>h</sup> ph<sup>h</sup>ng T<sup>h</sup>nh Cao B<sup>h</sup>ng</b>	Cao b <sup>h</sup> ng	Thu th <sup>h</sup> ép h <sup>h</sup> y <sup>h</sup> ot thanh b <sup>h</sup> nh nh <sup>h</sup> on

21	<p><b>Liên kết với sản xuất vụ ô nhiễm</b>          (Ghi rõ tên và sản xuất hoặc ngành nghề sở hữu kết quả nghiên cứu tham gia vào quá trình thực hiện vụ nêu rõ nội dung công việc thực hiện trong ô nhiễm)</p> <p>Viễn Võ Sinh Dịch tổ Trung -ng, Bé Y tổ lụ c- quan võ sinh phđng đpch Quèc gia sĩ lụ n- i thu thếp c, c mếu bõnh phêm vụ tiõn hụnh xđt nghiõm b»ng c, c kũ thuết hiõn ôi</p> <p>C, c bõnh viõn vụ c, c Trung tøm Y tổ Dù phđng lụ n- i cung cêp bõnh phêm vụ ô-íc h-êng c, c kõt xđt nghiõm cõng nh- nõu cã v"cxin ô ti"m phđng cho ng-êi đon sau nuy.</p>
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22	<p><b>Số người có mặt thực hiện ô nhiễm</b>          (Ghi ngành nghề của ông/gặp chính thức tất cả các tác giả chỉ trực tiếp tham gia ô nhiễm, không quá 10 người)</p>		
TT	Hà vụ t"n	C- quan c«ng t, c	Tu lĩ thêi gian lụm viõc cho ô nhiễm/ dù , n
A	<b>Chỉ nhiệm ô nhiễm:</b>		
	PGS.TS. Nguyễn Trçn Hiõn	Viễn Võ sinh Dịch tổ Trung -ng	25%
B	<b>C, n bé tham gia nghiên cứu:</b>		
1	GS.TS. Tr-ng Uy"n Ninh	Viễn Võ sinh Dịch tổ Trung -ng	50%
2	TS. L" Quính Mai	Viễn Võ sinh Dịch tổ Trung -ng	25%
3	ThS. Tr-ng Thõa Th"ng	Viễn Võ sinh Dịch tổ Trung -ng	100%
4	BS. Ph"m Thp Bých Ngắc	Viễn Võ sinh Dịch tổ Trung -ng	100%
5	ThS. Nguyễn Thp Thu	Viễn Võ sinh Dịch tổ	100%



	Thầy	Trung -ng	
6	TS. Trần Thới Hinh	Bệnh viện Bạch Mai	25%
7	TS. Nguyễn Quang Huy	Viện Nhi Trung -ng	25%
8	ThS. Hoàng Quang Hinh	Trung tâm Y tế Dù phng Cao Bng	25%
9	TS. Trần Thị Ngắc	Bệnh viện Bạch Mai	25%
10	BS. Nguyễn Xuân Tr-êng	Trung tâm Y tế Dù phng Ling S-n	25%

### V. Kinh phí thực hiện ở tại vụ quản kinh phí

(Giới trình chi tiết xin xem phụ lục kèm theo)

S-n vP tYnh: Triệu  
Âng

2	Kinh phí thực hiện ở tại phân theo c,c khoản chi						
T	Nguồn kinh phí	Tăng sè	Trong ở				
			Thu <sup>a</sup> kho,n chuy <sup>a</sup> n m<n	Nguy <sup>a</sup> n,về t liêu, n"ng l-ìng	Thiốt bP, m,ymã c	Xoy dùng, sõa ch÷a	Chi kh, c
1	2	3	4	5	6	7	8
	<b>Tăng kinh phí</b>	6.570	170	1.400	5.000	0	0
	<b>Trong ở:</b>						
	Ngôn s, ch	1.570	170	1.272	128	0	0
	Hoa Kú						
	C,c nguồn vèn kh,c						
	- Tù cã	5.000	0	0	5.000	0	0
	- Kh,c .....		0	0	0	0	0

Hà Nội, ngày 12 tháng 03 năm 2008.

**Thủ tr-êng**  
**Quan chỉ trx ở tại**  
(Hã, t<sup>a</sup>n, ch÷ ký vụ ởng  
đều)

**Chỉ nhiệm ở tại**  
(Hã, t<sup>a</sup>n vụ ch÷ ký)

PGS.TS. Nguyễn Trọng Hiếu

....., ngày ..... tháng ..... năm 2008  
 Phê duyệt của thủ trưởng cơ quan quản lý

Dù tãan kinh phí @0 tui/ Dù ,n nghi^an cõu khoa hãc

S-n vi. Triệu @ảng

TT	Néi dung c,c kho^n chi	Tạng sè		Nguồn vèn		
		Kinh phí	Tû lã (%)	NS Hoa Kú	Tủ cã	Kh,c
1	Thu^ khãan chuy^an m<n	170	2,6	170		
2	Nguy^an vEt liõu, n'ng l-ing	1.272	19,4	1.272		
3	Thiõt bP m,y mắc chuy^an ðõng	5.128	78,0	128	5.000	
4	Xõy ðùng, sõa ch÷a nhá	0	0			
5	Chi kh,c	0	0			
<b>Tạng cęg</b>		<b>6.570</b>	<b>100,0</b>	<b>1.570</b>	<b>5.000</b>	

Giãi trxn^h c,c kho^n chi  
 (Triệu @ảng)

Kho^n I: Thu^ kho,n chuy^an m<n

SV. Triệu @ảng

TT	Néi dung thu^ khan	Tạng kinh phí	Nguồn vèn		
			NS Hoa Kú	Tủ cã	Kh,c
1	Thu^ kho,n chuy^an m<n	150	150	0	0
2	Thu^ c<ng vEn chuyõn trong n-íc	20	20		

3	Vễn chuyển ra n-íc ngãi				
	<b>Tăng cng</b>	<b>170</b>	<b>170</b>	<b>0</b>	<b>0</b>

Khoản II: thiế b m.y mắ

SV. Triệu ăng

TT	Néi dung	SV @o	Sè l-íng	S-n gi.	Thnh tiền	Nguán vèn		
						Hoa Kú	Tù cã	Kh,c
1	Tả l'nh s@u	Lit	01	128,0	128,0	<b>128,0</b>	0	0

Khoản iIII: Nguy^n vệt liêu vụ n'ng l-íng  
(Hãa chết, đông cô, thu^ xe, s.ch...)

SV. Triệu ăng

TT	Néi dung chi	S-n v@ @o	Sè l-íng	S-n gi. (@)	Thnh tiền	Nguán vèn		
						Hoa Kú	Tù cã	Kh,c
1	Thu^ xe vễn chuyển trong n-íc	Chuyển	110	1,2	132,0	132,0	0	0
	Gõ ra n-íc ngãi	Chuyển	11	17.312000	190, 432	190, 432	0	0
2	Nguy^n vệt liêu							
	B-m ti^m	B-m	1600	3.940	6,304	6,304	0	0
	Kim ti^m	Kim	2000	1.144	2,288	2,288	0	0
	G'ng tay	ChiỐc	2.400	880	2,112	2,112	0	0
	G'c	Thíng	2	3,760000	7,520	7,520	0	0
	Giếy Iode	Hép	20	240.000	4,800	4,800	0	0
	Doy Garose	Hép	02	5.712.000	11,424	11,424	0	0
	Chết khô trỉng	Chai	24	158.000	3,792	3,792	0	0
	Hép @ùng @Cu c<n	Hép	3000	9.232	27,696	27,696	0	0
	Aã v< trỉng	ChiỐc	30	83,733	2,512	2,512	0	0
	KhEu trang	ChiỐc	400	8.880	3,552	3,552	0	0
	Kýnh b@o hé	C,i	12	112.000	1,344	1,344	0	0
	Hãa chết EDTA	Lit	01	2.400.000	2,400	2,400	0	0
	Hép b@o qu@n	Hép	25	256.000	6,400	6,400	0	0
	Hép vễn chuyển	Hép	5	1.504.000	7,520	7,520	0	0
	Tube 15- 50 ml	Tube	500	27.200	13,600	13,600	0	0
	Tube Ependorf	Hép	2	176.000	0,352	0,352	0	0
	Kits ELISA	Kits	20	11.120.000	222,400	222,400	0	0
	Kits IFA	Kits	20	7.200.000	144,000	144,000	0	0
	Hãa chết ph@n lễp	Kits	10	4.800.000	48,000	48,000	0	0
	Kits QIAamp viral	Kits	4	11.872.000	47,488	47,488	0	0
	Kits RT-PCR/	Kits	20	6.272.000	125,440	125,440	0	0

	PCR							
	Kits Purification	Kits	04	6.048.000	24,193	24,193	0	0
	Gen Electrophoresis	Kits	10	1.440.000	14,400	14,400	0	0
	Primers c,c lăai	Măi	200	368.000	73,600	73,600	0	0
	Sequencing		2000	32.000	64,000	64,000	0	0
	Fotal caff serum	Lit	10	740.000	7,400	7,400	0	0
	C,c hăa chÊt khac	lýt	30	2.000.000	60,000	60,000	0	0
	TÊm nhũa 96 giÕng	Hép	10	1.520.000	15,200	15,200	0	0
	<b>Tăng céng</b>				<b>1.272,0</b>	<b>1.272,0</b>		

Appendix V: Agreements between the UH and NIHE through August 15, 2009.

## MINISTRY OF HEALTH

SOCIALIST REPUBLIC OF VIETNAM  
Independence- Freedom- Happiness

June 06, 2008

To: Duane J. Gubler, ScD, FIDSA, FAAAS  
Director, Asia-Pacific Institute of Tropical Medicine and Infectious Diseases  
Professor & Chair, Department of Tropical Medicine, Medical Microbiology and  
Pharmacology  
John A. Burns School of Medicine  
651 Ilalo Street, BSB 3rd Floor  
Honolulu, HI 96813

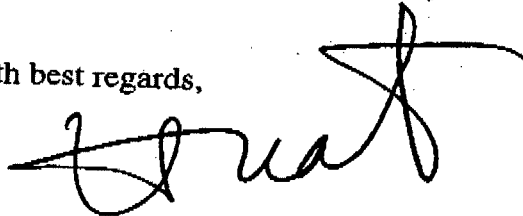
Dear Dr. Gubler,

Thank you very much for informing us the collaborative project between you and NIHE on Collaborative Research & Training Program Using Advanced Technologies to Detect Emerging Infectious Diseases of Southeast Asia.

We highly appreciate your efforts to collaborate with Vietnam for strengthening our capacity to control emerging infectious diseases. Following our regulation, all international collaborative project proposals should go through review procedures before it is approved officially by the MoH. It is firstly reviewed by ethical and scientific committees of the local institution. Then it is submitted to the MoH and is reviewed again by MoH related departments and finally by the MoH committee. These procedures just make sure that all projects are following our management regulations and for better research quality. Your collaborative research proposal with NIHE was submitted to MoH and is going through the review process of MoH. It will get approval in next few weeks. I strongly support this project and do not expect any reason for disapproval. For the time being, I would like to ask you to extend the period of performance for Phase I of the project until July 15, 2008.

Once again, I would like to thank you very much for your collaboration.

With best regards,



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Prof. Trinh Quan Huan  
Vice Minister of Health

Minister of Health  
NIHE  
Document No. 645

SOCIALIST REPUBLIC OF VIETNAM  
Independence-Freedom-Happiness

Dear: Professor Trinh Quan Huan  
Vice minister of Health

The National Institute of Hygiene and Epidemiology (NIHE) had turned in phase I of the proposal “syndromic surveillance system will be established to monitor febrile illness caused by infectious agents in North Vietnam” to the minister of health. This is collaboration between NIHE and UH-APITMID regard to “Collaborative Research & Training Program Using Advanced Technologies to Detect Emerging Infectious Diseases of Southeast Asia”

The goal of this proposal is screen and detect new infectious agents from serum of febrile patient (phase I)

Proposal time frame: 1 year from the date the proposal is approved

Sentinel surveillance sites of the proposal are NIHE, Bach Mai Hospital, Hanoi, Long Son Provincial Hospital, Viet Tiep City Hospital in Hai Phong, Hoa Binh District and Cao Bang.

Total expense: 99,432.00 Dollars

Due to the extend time taken during discussions regarding the protocol of how to carry out the proposal, we are running of time which is given by the funding agency. Therefore, NIHE suggests Vice Minister of Health to write a letter of support to University of Hawaii-APITMID.

Logo of NIHE

Nguyen Tran Hien

*Hà Nội, ngày 27 tháng 6 năm 2008*

**QUYẾT ĐỊNH**

Về việc phê duyệt tiếp nhận dự án “Bước đầu sàng lọc phát hiện vi rút mới gây bệnh cho người ở một số khu vực tại Việt Nam”, do tổ chức Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, Trường Đại học Tổng hợp Hawaii, Hoa Kỳ, viện trợ

**BỘ TRƯỞNG BỘ Y TẾ**

Căn cứ Nghị định số 188/2007/NĐ-CP ngày 27/12/2007 của Chính phủ quy định về chức năng, nhiệm vụ, quyền hạn và cơ cấu tổ chức của Bộ Y tế;

Căn cứ qui định tại Quyết định số 64/2001/QĐ-TTg ngày 26/4/2001 của Thủ tướng Chính phủ về Quy chế quản lý và sử dụng viện trợ Phi chính phủ nước ngoài;

Căn cứ qui định tại Quyết định số 1829/2002/QĐ-BYT ngày 17/5/2002 của Bộ trưởng Bộ Y tế về quản lý và sử dụng viện trợ Phi chính phủ nước ngoài trong ngành Y tế;

Xét Công văn số 634/VSDTTU ngày 04/6/2008 của Viện Vệ sinh dịch tễ TW đề nghị Bộ Y tế phê duyệt tiếp nhận dự án Bước đầu sàng lọc phát hiện vi rút mới gây bệnh cho người ở một số khu vực tại Việt Nam, do tổ chức Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, Trường Đại học Tổng hợp Hawaii, Hoa Kỳ viện trợ;

Theo đề nghị của Vụ trưởng Vụ Kế hoạch-Tài chính, Bộ Y tế,

**QUYẾT ĐỊNH:**

**Điều 1.** Phê duyệt tiếp nhận dự án Bước đầu sàng lọc phát hiện vi rút mới gây bệnh cho người ở một số khu vực tại Việt Nam, do tổ chức Asia-Pacific Institute of Tropical Medicine and Infectious Diseases (Viện Y học nhiệt đới và Bệnh nhiễm trùng châu Á - Thái Bình Dương) – Trường Y J.A. Burns, Trường Đại học Tổng hợp Hawaii, Hoa Kỳ, viện trợ với các nội dung chính như sau:

1. Tên dự án: Bước đầu sàng lọc phát hiện vi rút mới gây bệnh cho người ở một số khu vực tại Việt Nam.
2. Cơ quan chủ quản: Bộ Y tế.
3. Đơn vị chủ dự án: Viện Vệ sinh dịch tễ TW.
4. Địa điểm thực hiện: một số Bệnh viện và Trung tâm Y tế dự phòng tại các tỉnh và thành phố: Hà Nội, Lạng Sơn, Hải Phòng, Hoà Bình, Cao Bằng.
5. Mục tiêu dự án:
  - a) Mục tiêu chung: Phát hiện và dự báo các vi rút mới xuất hiện ở Việt Nam và các nước trong khu vực Đông Nam Á để có sinh phẩm chẩn đoán và vắc xin phòng bệnh.
  - b) Mục tiêu cụ thể:
    - Sàng lọc phát hiện những vi rút mới xuất hiện ở bệnh nhân mắc bệnh truyền nhiễm do vi rút,



- Bước đầu xây dựng hệ thống giám sát, xét nghiệm phát hiện vi rút mới gây bệnh ở người.
- 6. Tổng ngân sách dự án: 99.432 USD (chín chín nghìn bốn trăm ba hai đôla Mỹ), do tổ chức Asia-Pacific Institute of Tropical Medicine and Infectious Diseases viện trợ.
- 7. Thời gian thực hiện: 01 năm (2008 – 2009).

**Điều 2.** Viện Vệ sinh dịch tễ TW chịu trách nhiệm tiếp nhận và thực hiện dự án được phê duyệt tại Điều 1 của Quyết định này theo đúng quy định hiện hành của Nhà nước về quản lý và sử dụng viện trợ phi chính phủ nước ngoài, đảm bảo đúng mục tiêu, tiến độ, và thực hiện đầy đủ chế độ báo cáo, thanh quyết toán viện trợ theo qui định. Viện Vệ sinh dịch tễ TW thực hiện dự án theo nội dung văn kiện dự án phê duyệt kèm theo quyết định này.

**Điều 3.** Quyết định này có hiệu lực kể từ ngày ký ban hành.

**Điều 4.** Các Ông Vụ, Cục trưởng các Vụ, Cục: Kế hoạch-Tài chính, Hợp tác Quốc tế, Khoa học - Đào tạo, Y tế dự phòng và Môi trường, và Thủ trưởng các đơn vị có liên quan chịu trách nhiệm thi hành quyết định này.

**Nơi nhận:**

- Như Điều 4;
- Bộ trưởng (để báo cáo);
- Bộ Kế hoạch & Đầu tư;
- Bộ Tài chính;
- Liên hiệp các tổ chức hữu nghị VN;
- Lưu: VT, KH-TC5.

**KT. BỘ TRƯỞNG**

**THỦ TRƯỞNG**



**Trịnh Quân Huấn**

**LIÊN HIỆP CÁC TỔ CHỨC  
HỮU NGHỊ VIỆT NAM**

Số: 11.24.../LH-PA

(T/y: Góp ý về dự án do Trường Y thuộc ĐH  
Hawaii-Mỹ cho Viện Vệ sinh dịch tễ Trung  
ương- Bộ Y tế).

**CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM  
Độc lập - Tự do - Hạnh phúc**

Hà Nội, ngày 16 tháng 6 năm 2008

Kính gửi: Bộ Y tế (Viện Vệ sinh Dịch tễ Trung ương).

Phúc công văn số 638/VSDTTU ngày 05/6/2008 của Viện Vệ sinh Dịch tễ Trung ương - Bộ Y tế về việc tiếp nhận dự án “Bước đầu sàng lọc phát hiện virus mới gây bệnh cho người tại một số khu vực ở Việt Nam”, trị giá 99.432 đô la Mỹ do Viện nghiên cứu các bệnh truyền nhiễm và các bệnh nhiệt đới tại châu Á-Thái Bình Dương, thuộc Trường Đại học Y J.A. Burns thuộc Đại học Tổng hợp Hawaii-Honolulu, Hoa Kỳ tài trợ, Liên hiệp các tổ chức hữu nghị Việt Nam (Liên hiệp Hữu nghị) có ý kiến như sau:

- Dự án nêu trên nhằm mục tiêu nâng cao năng lực nghiên cứu, phát hiện và điều trị dự phòng các bệnh truyền nhiễm và các bệnh nhiệt đới tại Việt Nam, phù hợp với chủ trương và mục tiêu tổng thể của ngành y tế Việt Nam. Về nguyên tắc, Liên hiệp Hữu nghị ủng hộ việc Viện Vệ sinh Dịch tễ Trung ương tiếp nhận và triển khai dự án này, song cần được Bộ Y tế phê duyệt theo Quy chế Quản lý và Sử dụng viện trợ phi chính phủ nước ngoài ban hành kèm theo Quyết định số 64/2001/QĐ-TTg ngày 26/4/2001 của Thủ tướng Chính phủ.

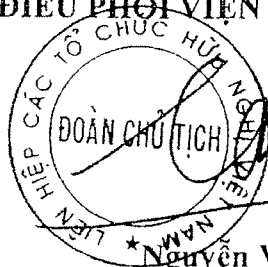
- Đề nghị Bộ Y tế chỉ đạo Viện Vệ sinh Dịch tễ Trung ương và các đơn vị liên quan hướng dẫn Viện nghiên cứu các bệnh truyền nhiễm và các bệnh nhiệt đới tại châu Á-Thái Bình Dương, thuộc Trường Đại học Y J.A. Burns thuộc Đại học Tổng hợp Hawaii-Honolulu, Hoa Kỳ liên hệ với Ủy ban Công tác về các tổ chức phi chính phủ nước ngoài (thông qua Ban Điều phối viện trợ nhân dân-PACCOM) để tiến hành thủ tục đăng ký hoạt động tại Việt Nam theo Quy chế về hoạt động của các TCPCPNN tại Việt Nam ban hành kèm theo Quyết định 340/TTg ngày 24/5/1996 của Thủ tướng Chính phủ.

Liên hiệp Hữu nghị xin trao đổi để Quý Cơ quan được biết./..

**KT. CHỦ TỊCH  
TỔNG THƯ KÝ KIỂM TRƯỞNG BAN  
BAN ĐIỀU PHỐI VIỆN TRỢ NHÂN DÂN**

Nơi nhận:

- Như trên;
- Vụ HTQT (Bộ Y tế);
- Lưu VPLH, PACCOM (BM)/.



Nguyễn Văn Kiên

PACCOM

Document No. 1184

SOCIALIST REPUBLIC OF VIETNAM  
Independence-Freedom-Happiness  
Hanoi, June 16, 2008

To: Minister of Health

According to document number 638/NIHE dated on May 6, 2008 from The National Institute of Hygiene and Epidemiology, NIHE had turned in phase I of the proposal “syndromic surveillance system will be established to monitor febrile illness caused by infectious agents in Northern Vietnam” to the minister of health. This is collaboration between NIHE and UH-APITMID regard to “Collaborative Research & Training Program Using Advanced Technologies to Detect Emerging Infectious Diseases of Southeast Asia” This phase has the budget of \$99.432 which is funded by the UH-APITMID. The PACCOM had suggested as follow:

The goals of the above proposal are to establish sentinel surveillance for early detection of tropical disease, to investigate the ecology and epidemiology of pathogens in Vietnam. These goals are well fitted with the general goals of the Vietnam Minister of health. In principle, PACCOM supports the collaboration of NIHE with UH-APITMID to carry out this proposal. However, this project need to be reviewed by Minister of Health for the usage and management of the funding as stated in the regulation number 64/2001 which was issued by Prime Minister.

Suggest Minister of Health to direct NIHE, Vietnamese agencies which are related to this project and UH-APITMID to contact the committee of PACCOM for registration to carry out research activities in Vietnam as stated in regulation number 340 which was issued by Prime Minister.

Logo of PACCOM

Chairman

Nguyen Van Kieu

**AMENDMENT NO. 1**  
TO THE AGREEMENT BETWEEN  
THE RESEARCH CORPORATION OF THE UNIVERSITY OF HAWAII  
AND  
VIETNAM, NATIONAL INSTITUTE OF HYGIENE AND EPIDEMIOLOGY  
(VN NIHE)

This Amendment No. 1, is made and entered into as of 29 October 2007 by and between the Research Corporation of the University of Hawaii (hereinafter "RCUH"), in behalf of ADV TECH ASIA-PACIFIC DISEASES, and the VIETNAM, NATIONAL INSTITUTE OF HYGIENE AND EPIDEMIOLOGY (VN NIHE) (hereinafter "Subcontractor").


RCUH and the Subcontractor entered into that certain Agreement dated 06 February 2008 whereby the Subcontractor agreed to provide the services described in the Agreement. RCUH and the Subcontractor mutually agree to modify the Agreement as follows:

Amend Section 1, page 1 to read as follows:

3. Period of Agreement and Amount: The period of performance of this Agreement shall be October 29, 2007 through August 15, 2009, as listed in the Project Budget which is attached hereto as Attachment 3 and made a part hereof by reference, and provide, further, that expenditure from monies to provided by RCHI pursuant to this Agreement shall not exceed the total amount of \$99,432.00.

It is understood that this Amendment is supplemental to the Agreement entered into dated October 29, 2007, and that all terms, conditions and provisions of that Agreement remain in full force and effect unless specifically modified, altered or changed herein.


SUBCONTRACTOR

  
\_\_\_\_\_  
Date: July 22, 2008

RESEARCH CORPORATION  
OF THE UNIVERSITY OF HAWAII

\_\_\_\_\_  
Date: \_\_\_\_\_

PROJECT AUTHORITY

  
\_\_\_\_\_  
Date: 25 July, 2008

AGREEMENT BETWEEN  
THE RESEARCH CORPORATION OF THE UNIVERSITY OF HAWAII

and

Viet Nam, National Institute of Hygiene and Epidemiology

THIS AGREEMENT, entered into this 29th day of October, 2007 by and between The Research Corporation of the University of Hawaii, hereinafter called "RCUH", a governmental agency of the State of Hawaii, whose post office address is 2800 Woodlawn Drive, Suite 200, Honolulu, Hawaii 96822, for the benefit of Department of Tropical Medicine, Medical Microbiology & Pharmacology, hereinafter called the "Project", and VN NIHE, hereinafter called "SUBCONTRACTOR", whose business address and tax identification number are as follows:

Vietnam's National Institute of Hygiene and Epidemiology (NIHE)

Address: 1 Yersin Street, Hai Ba Trung District, Hanoi

Tax ID: Not Applicable - see Tax Clearance P & P 2.218 SL; Sec 103-53, HRS

WITNESSETH THAT:

WHEREAS, the Project and the Funding Agency are parties to a Grant/Contract Number W81XWH-07-2-0073 and the CFDA number, if applicable, 12.42 entitled "Advanced Technologies Addressing Asia-Pacific Infectious Diseases", a copy of which is attached hereto as Attachment 1 and by reference made a part hereof; and

WHEREAS, the Project has requested RCUH to provide administrative services for their benefit in support of the Grant/Contract referenced above, in accordance with the Internal Agreement between UH and RCUH, and

WHEREAS, RCUH for the benefit of the Project and the Principal Investigator of said grant/contract will provide financial support to the SUBCONTRACTOR to provide the Services detailed in the Statement of Work attached hereto as Attachment 2; and

WHEREAS, RCUH desires to compensate the SUBCONTRACTOR for their Subcontract work in accordance with the applicable budget attached hereto as Attachment 3 and by reference made a part hereof; NOW, THEREFORE, in consideration of the mutual covenants and agreements hereinafter contained, the parties hereto agree:

1. The SUBCONTRACTOR agrees to perform the necessary services for the implementation and completion of that portion of the contract above referred to as relates to the Project.

2. The SUBCONTRACTOR agrees to conform to all federal rules and regulations that may apply to the conduct and performance of this Agreement.

3. Period of Agreement and Amount: The period of performance of this Agreement shall be October 29, 2007 through August 15, 2008, as listed in the Project Budget which is attached hereto as Attachment 3 and made a part hereof by reference, and provided, further, that expenditures from monies to be provided by RCUH pursuant to this Agreement shall not exceed the total amount of \$99,432.00.

4. Method of Payment: RCUH shall reimburse the SUBCONTRACTOR for expenses incurred by the project on a monthly basis. Such reimbursement will be made in accordance with the submission or completion of required deliverables, monthly billings prepared and submitted by the SUBCONTRACTOR, broken down by expense categories reflected in the budget, and must be consistent with the rules and regulations contained in Attachment 1 of this subcontract. Failure to submit invoices on a timely basis may result in delayed reimbursement. RCUH may withhold reimbursement payments if quarterly financial reports are not submitted on a timely basis or if reported cost sharing data is not current or proportional to the annual estimated obligation.

5. Audit Requirements: The provisions of Office of Management and Budget (OMB) Circular A-133, "Audits of Institutions of Higher Learning and Other Nonprofit Institutions," apply to this contract. The SUBCONTRACTOR agrees to submit a copy of its annual A-133 audit and corrective actions to RCUH within one month of completion and release of the audit report. Failure to submit the audit report and corrective actions may result in delayed reimbursement to SUBCONTRACTOR for costs incurred under the contract.

6. Examination of Records: The SUBCONTRACTOR agrees that the U.S. Comptroller General, University of Hawaii, RCUH, or any of their duly authorized representatives shall have access to, and the right to inspect or audit any directly pertinent books, documents, papers and records of the SUBCONTRACTOR involving transactions related to this Agreement.

7. Termination: The RCUH shall have the privilege, with or without cause, to cancel or annul this Agreement at any time upon written notice given thirty (30) days in advance of such termination.

8. Disallowed Costs: The SUBCONTRACTOR shall be responsible for reimbursement to the prime SUBCONTRACTOR, UH, a sum of money equivalent to the amount of any expenditures disallowed should the funding agency or an authorized agency rule through audit exception or some other appropriate means that expenditures from funds allocated to the SUBCONTRACTOR for direct and/or indirect costs were not made in compliance with the applicable cost principles, regulation of the funding agency, or the provisions of this Subcontract.

9. Disputes: Any disputes concerning a matter of fact arising under this Agreement, which is not disposed of by mutual agreement within thirty (30) calendar days, shall be determined by the Executive Director of RCUH, or said Executive Director's designated representative who shall reduce the decision to writing and mail or otherwise furnish a copy of the decision to the SUBCONTRACTOR. Pending final decision of such a dispute, the SUBCONTRACTOR shall proceed diligently with the performance of services under this Agreement in accordance with RCUH's request.

10. Federal Funds: If this Agreement is payable in whole or in part from federal funds, the SUBCONTRACTOR agrees that, as to the portion of the compensation under this Agreement to be payable from federal funds, the SUBCONTRACTOR shall be paid only from such federal funds received from the federal government, and shall not be paid from any other funds.

11. Ownership Rights and Copyright: RCUH shall have complete ownership of all material, both finished and unfinished, which is developed, prepared, assembled, or conceived by the SUBCONTRACTOR pursuant to this Agreement, and all such material shall be considered "works made for hire". All such material shall be delivered to the RCUH upon expiration or termination of this Agreement. RCUH, at its discretion, shall have the exclusive right to copyright any product, concept, or material developed, prepared, assembled, or conceived by the SUBCONTRACTOR pursuant to this Agreement. The SUBCONTRACTOR, however, reserves the right to use thereafter any ideas and techniques that may be developed during the performance of this Agreement.

12. Governing Law: The validity of the Agreement and any of its terms or provisions, as well as the rights and duties of the parties to this Agreement, shall be governed by the laws of the State of Hawaii. Any action at law or in equity to enforce or interpret the provisions of this Agreement shall be brought in a state court of competent jurisdiction in Honolulu, Hawaii.

13. Indemnification and Defense: SUBCONTRACTOR shall defend, indemnify, and hold harmless RCUH, the University of Hawaii, the State of Hawaii, the contracting agency, and their officers, employees, and agents from and against all liability, loss, damage, cost, and expense, including all attorneys' fees, and all claims, suits, and demands thereof, arising out of or resulting from the acts or omissions of the SUBCONTRACTOR or its employees, officers, agents, or SUBCONTRACTORS under this Agreement. The provisions of this paragraph shall remain in full force and effect notwithstanding the expiration or early termination of this Agreement.

14. Modifications of Agreement: Any modification, alteration, amendment, change, or extension of any term, provision, or condition of this Agreement shall be made only by written amendments to this Agreement, signed by both the SUBCONTRACTOR and RCUH.

15. Relationship of Parties; Independent SUBCONTRACTOR Status and Responsibilities:

a. In the performance of services, or delivery of goods, or both, required under this Agreement, the SUBCONTRACTOR is an "independent SUBCONTRACTOR," with the authority and responsibility to control and direct the performance and details of the work and services required under this Agreement; however, RCUH shall have a general right to inspect work in progress to determine whether, in RCUH's opinion, the services are being performed or the goods are being provided, or both, by SUBCONTRACTOR.

b. The SUBCONTRACTOR and the SUBCONTRACTOR's employees and agents are not by reason of this Agreement, agents or employees of RCUH for any purpose, and the SUBCONTRACTOR and the SUBCONTRACTOR's employees and agents shall not be entitled to claim or receive from RCUH any vacation, sick leave, retirement, workers' compensation, unemployment insurance, or other benefits provided to RCUH employees.

c. The SUBCONTRACTOR shall be responsible for the accuracy, completeness, and adequacy of its performance under this Agreement. Furthermore, the SUBCONTRACTOR intentionally, voluntarily, and knowingly assumes the sole and entire liability to the SUBCONTRACTOR's employees and agents, and to any individual not a party to this Agreement, for all loss, damage, or injury caused by the SUBCONTRACTOR, or the SUBCONTRACTOR's employees or agents in the course of their employment.

d. The SUBCONTRACTOR shall be responsible for payment of all applicable federal, state, and county taxes and fees which may become due and owing by the SUBCONTRACTOR by reason of this Agreement, including but not limited to (i) income taxes, (ii) employment related fees, assessments, and taxes, and (iii) general excise taxes. The SUBCONTRACTOR is responsible for obtaining all licenses, permits, and certificates that may be required in order to perform the Agreement.

e. The SUBCONTRACTOR is responsible for securing all employee-related insurance coverage for the SUBCONTRACTOR and the SUBCONTRACTOR's employees and agents that is or may be required by law, and for payment of all premiums, costs, and other liabilities associated with securing the insurance coverage.

16. Tax Clearances: In accordance with Section 103-53, Hawaii Revised Statutes, if the amount of this Agreement is \$25,000 or more, a tax clearance from the Director of Taxation, State of

Hawaii and the Internal Revenue Service is required before this subcontract can become effective. Tax clearances are also required prior to the release of final payment.

17. General Conditions: The terms, conditions, provisions, and special requirements of the Prime Grant/Contract will apply to this Agreement and are attached hereto as Attachment 1 and made a part hereof by reference.

18. Standards of Conduct Declaration. The Standards of Conduct Declaration by SUBCONTRACTOR, set forth in Attachment 4, is hereby made a part of this Agreement.

19. Federal Provisions. If federal grant funds are expended under this contract, the SUBCONTRACTOR shall comply with the applicable provisions of Attachment 32a. If federal contract funds are expended under this contract, the SUBCONTRACTOR shall comply with the applicable provisions of Attachment 32b, 32c, or 32d.

20. It is understood that this Agreement represents the sole and entire Agreement between the parties.



IN WITNESSETH WHEREOF, The SUBCONTRACTOR and The Research Corporation of the University of Hawaii have executed this Agreement at Honolulu, Hawaii on 10/29/07 with retroactive effect as of and from the date first above written.

RECOMMENDED BY:

  
PRINCIPAL INVESTIGATOR OR  
PROJECT AUTHORITY

Date: 22/12/07

EXECUTED BY:

SUBCONTRACTOR

Date: \_\_\_\_\_

THE RESEARCH CORPORATION OF  
THE UNIVERSITY OF HAWAII

Date: \_\_\_\_\_

## STANDARDS OF CONDUCT DECLARATION

For the purposes of this declaration:

"Employee" means any nominated, appointed, or elected officer or employee of the State, including members of boards, commissions, and committees, and employee under contract to the State or of the Constitutional Convention, but excluding legislators, delegates to the Constitutional Convention, justices and judges.

"Controlling interest" means an interest in a business or other undertaking which is sufficient in fact to control, whether the interest be greater or less than fifty per cent.

On behalf of VN NIHE, SUBCONTRACTOR, the undersigned does declare, under penalty of perjury, as follows:

1. SUBCONTRACTOR (is) (is not) a legislator or an employee or a business in which a legislator or an employee has a "controlling interest".
2. SUBCONTRACTOR has not been assisted or represented by a legislator or employee for a fee or other compensation to obtain this Agreement and will not be assisted or represented by a legislator or employee for a fee or other compensation in the performance of the Agreement, if the legislator or employee had been involved in the development or award of the Agreement.
3. SUBCONTRACTOR has not been assisted or represented for a fee or other compensation in the award of this Agreement by a RCUH employee, or in the case of the Legislature, by a legislator.
4. SUBCONTRACTOR has not been represented or assisted personally on matters related to the Agreement by a person who has been an employee of the RCUH within the preceding two years and who participated while in state office or employment on the matter with which the contract is directly concerned.
5. SUBCONTRACTOR has not been represented or assisted on matters related to the Agreement, for a fee or other consideration by an individual who, within the past twelve months, has been a RCUH employee.
6. SUBCONTRACTOR has not been represented or assisted in the award of this Agreement for a fee or other consideration by an individual who, (a) within the past twelve months, served as a RCUH employee, and (b) participated while an employee on matters related to this Agreement.

SUBCONTRACTOR understands that the Agreement to which this document is attached is voidable on behalf of the RCUH if this Agreement was entered into in violation of any provision of chapter 84, Hawaii Revised Statutes, commonly referred to as the Code of Ethics, including the provisions which are the source of the declarations above. Additionally, any fee, compensation, gift, or profit received by any person as a result of a violation of the Code of Ethics may be recovered by RCUH.

Dated: December 20, 2007

SUBCONTRACTOR

\_\_\_\_\_  
By Director, VN NIHE

\_\_\_\_\_  
Its (Title)

## Appendix VI: Trip Reports to Vietnam.

TRIP REPORT  
Hanoi, Vietnam  
September 8 – 18 2007

**Action Items:**

1. Prepare new request for IRB exemption letter from the NIHE. It will need to include:
    - a. Budget of \$75K - \$100K – *IRB exemption letter must be provided before UH can release money and NIHE cannot execute the project until it is approved by MoH. The project is under \$500K; therefore, the chain of approval is shorter and will not require outside entity review.*
      - i. Courier costs based on weekly runs from several regions
      - ii. International transfer w/new procedures
      - iii. Collections of samples for (1) Labor and (2) Kits, equipment
      - iv. Screening costs (1) Lab expenses and (2) Labor
      - v. Technical Labor costs
      - vi. # of samples (projected) for 5 collection sites :
        1. Bach Mai
        2. Lang San – Provincial Hospital
        3. Viet Tiep Friendship Hospital – City Hospital in Haiphong
        4. Hoabinh – District Hospital (Preventive Medicine)
        5. Cao Bang – District Hospital (Preventive Medicine)
      - vii. 11 month period of collection to cover “seasonal” effects
      - viii. Protocol as appendices
    - b. Translated into VN format
  2. Bach Mai Hospital :
    - a. Work with Neurology department to ensure incoming cases of Meningoencephalitis are identified
    - b. Facilitate Dengue Vaccine production by NIHE
      - i. Already producing Polio, Hep.
      - ii. Rabies vaccination production stopped due to side effects
    - c. Work w/Bach Mai to develop protocols to do autopsies on patients who die following viral syndrome. *(talked with Mai early in the day about this but did not mention in the Bach Mai meeting)*
  3. Blood bank study to screen for Dengue viruses. Imminent to perform while currently experiencing a DNV outbreak. Look to publish findings.
  4. Meet with Army Hospital 103 (Hapong area) and 108 – both in Hanoi
  5. MoU w/HSPH – Dr. ANH will email Ginger Hendee a sample.
  6. Next trip, arrange meeting with Dr. Ming of VAST
  7. Contacts to f/u:
    - a. LTC Jerome Kim
    - b. Mr. Phil Easterman at East West Center
    - c. CDC, HHS, etc.
  8. November quarterly report – Begin work immediately to have completed by October 5, 2007.
  9. Check on Purchase Order with Avis Lam.
-

## Trip Events:

### Monday – September 10, 2007

- 0830 – Met with Professor **Truong Uyen NINH**, Dr. Le Thi Quynh **MAI** & Thang at NIHE. Dr. **Mai** is working with Dengue and is the Head, Avian Influenza Department. All of which are prior students of Dr. Duane Gubler. Dr. Gubler iterated the need to look for:
- Viral syndromic diseases i.e. Dengue-like, etc. in humans via blood samples
  - Hemorrhagic diseases
  - Neurological syndromes
  - Respiratory Illnesses
  - Patients who die after febrile illnesses

**Note:** *Autopsies are being performed Bach Mai and Dr. Gubler noted possible future opportunity for UH pathologist to work with Bach Mai pathologist.*

- 0900 – **Pham Ngoc DINH, M.D. Ph.D** (*Vice Director, NIHE*): Dr. Gubler explained the project and the vision of a possible collaboration with Duke University, Singapore, UH and CDC. Emphasized the importance of training and collaborative relationships throughout the region. Dr. Gubler asked about contacts in Dali, China and Dr. Dinh does not have any at this time. Dr. Dinh mentioned students who are in place at NIHE from Japan and Paris.

- 1000 – BACH MAI Hospital Director – **Bs.Ts. Tran Thuy HANH** (*wife of the new MoH*) – After the hospital was refurbished, focus is on Internal Medicine and includes pediatrics.
- Beds – 10K patients daily in VN; about 5K admitted daily
    - 100 overall
    - ICU – 7
    - General – 14
    - HIV – 14
    - Hepatitis
  - Swine Streptococcus – 4 cases in humans; more in mid-VN (*believed to be coming from China*)
  - Most common admission is “internal medicine” which includes ID.
  - Do not have an isolation room with negative pressure.
  - H5N1 samples go to NIHE & to lab in Bach Mai. Screened at Bach Mai w/Eliza and confirmed at NIHE by PCR & Eliza
  - Dr. Gubler talked about CDC relationship and UH department, regional network, etc.
  - Met with **Ts. Bs. Trinh Thi NGOC** (Vice Director of Infectious Disease Department, Bach Mai Hospital)
  - Met with Rickettsia Specialist: **Pham Thi Thanh THUY** and her colleague who works on HIV, **Do Duy YUONG, MD**
  - Dr. Thuy explained her 2001-2003 study with Typhus. Dr. Gubler talked about need to get more accurate screening and identification, etc. IAF non-specific
- 1130 – Met with **Nguyen Tran HIEN, M.D.** (*Director, NIHE*) & Professor Truong Uyen NINH, PhD, Director, NIHE. Dr. Hien will submit request for IRB Exemption.
- Dr. Gubler talked about the project, vision and regional network and opportunities to build substantial partnerships, etc.

- Dr. Hien was polite, but stated that the protocol had to be rewritten to clarify exactly what we want to do, how and where before he will present it to the IRB committee for consideration of exemption—he emphasized that we need to spell out in detail. He was not very encouraging.

1:00 – Lunch with Dr.'s Hien and Ninh and Thang.

3:00 – HSPH – Met with the Dean of HSPH, **Le Vu ANH, MD, MSc, PhD** and his assistant, **Tran Huu BICH MD, PHD**

- Just returned from Cambodia
- Students from Paris, Japan and Europe
- Will be traveling to Europe (Sweden is the mtg location) this month
- New school – ground breaking ceremony early next year
- Water sensor project – presenting challenges to install, damaged, etc.
- Talked about relationship w/Dr. Minh of VAST (*VN Science and Technology... ministerial level position*)
- MOU with HSPH/UH

1830 – Dinner/meeting with **Dr. HIEN, Director of NIHE**, Dr. Vu Sinh NAM, his wife, Ninh, Thang, Dr. Gubler, Bobbie and Ginger Hendee. (@ San Ho)

## **Tuesday – September 11, 2007**

1039 – Haiphong. Visited the NIMM – Vietnam National Institute of Maritime Medicine. Dr. *Nguyen Truong Son* is interested and open to collaborative research. His primary interest is with the Maritime relationships and nearby island cooperatives. He is working with various European countries and looks to expand those relationships where possible. The new facility is due to break ground next year. New hyperbaric chamber (monotube w/ability to hold one healthcare provider) is being installed by company from Australia. This will be 1 or 2 hyperbaric chambers in all of Vietnam. Current facility is outpatient with 10 inpatient beds with EEG, Ultrasound, X-ray and basic Laboratory capabilities. Well staffed and enthusiastic about future endeavors building the NIMM. 10-15 fisherman daily—mostly work related injuries and various other common illnesses. Not a lot of infectious diseases outside of STDs. Patients are referred to the main hospital in Haiphong. While this was an instructional visit, collaboration with this hospital is not part of the ATAAPED project.

1351 – City Hospital in Haiphong; Met with **Dr. Ngo Viet Hung**; Chairman of Infectious Disease. Thang translated our vision and goals and asked for support in the project. The doctor responded positively to the project. He has 2 doctors who train students and treat patients. No SARS in 2004/2005. He said he is seeing cases of Swine Streptococcus that are coming from local sources. They discussed cases of hearing loss associated with encephalitis. ... *I could not understand the rest of the conversation.* Dr. Ngo Viet Hung said he sees approximately 10 cases/annually with hemorrhagic fever – like illnesses and they are negative for Dengue Virus and zero fatal outcomes. He is seeing Japanese Encephalitis in adults but not confirmed. Adult encephalitis is around 50-60 cases annually that are not confirmed. We suggested that samples to be confirmed at NIHE. He also mentioned a study with hantavirus was started about four years ago. Closest hospital is 12KM. Approximately 2,000 visits per year. Area of coverage is 32KM radius. Many patients go directly to the district hospital.

Note: Dr. Gubler noted this could be a good place to put a liquid nitrogen refrigerator or use dry ice.

**Wednesday – September 12, 2007**

0800 – Meeting w/Thang; East-West Center Conference at Melia; setup meeting/dinner for Friday night.

0900 – Ginger Hendee met with the wives while Dr. Gubler and others attended conference.

**Thursday – September 13, 2007**

Conference during the day

1000 – Hung hosted a day in the country – ceramic factory, etc.

1900 – Dinner /Meeting with Dr. Trinh Quan Huan, Vice Minister of Health and long friend of Dr. Gubler, Dr. Vu Sinh Nam, Dr. Nguyen Tran Hien, Director of NIHE, Professor Truong Uyen Ninh, Dr. Gubler, Bobbie and Ginger Hendee. (@ Japanese restaurant)

**Friday – September 14, 2007**

0815 – Dr. Gubler and Ginger Hendee met with Dr. Hien, Director NIHE, Professor Truong Uyen Ninh at NIHE. He needs more information in order get IRB approval. He needs time periods, methods and out year budget and plans, etc. The budget needs to stay under \$500K. We assured him that the budget is well under \$500K that it is around \$75K.

0840 – While waiting for transportation to Lang Son, Thang explained that Dr. Hien has new requirements.

1208 – Dr. Gubler, Ginger Hendee, Prof Ninh and Thang arrived at Lang Son, northern border of China. Met with the director of the Centre for Preventive Medicine, Dr. Nguyen Xuan Truong and Dr. Nguyen Minh Diep, Director of the laboratory. 70-80K population in city, ~700K in province and China is approximately 200KM from this location. 85% minorities, 220 communes, 11 cities and 10 hospitals in district with communes having 40-50 beds and clinics with 3-5 beds. They forward an annual report to NIHE. ~24 ID that are reported and most common are respiratory infections, GI and common cold. Last year they saw a few Korean hemorrhagic scrub typhus/JE. Cannot screen appropriately and they only screen for Hep B. They would like to begin collecting samples and have them screened and confirmed. Dr. Truong mentioned he would first need MOU between Preventive Medicine and the Medical Center before collection could begin.

1300 – Lunch w/Director and Dr. Diep.

1400 – A representative from the Centre for Preventive Medicine escorted us with her driver in an ambulance to the Chinese border. We visited two different entry points.

2000 – Dinner in nearby town. Returned by 2200.

**Saturday September 15, 2007** - Met with Dr. Vu Sinh NAM; Associate Professor & his wife Hung (Dr. Nam works at MoH in the Dept of Preventive Medicine and prior employee of NIHE and colleague of Dr. Gubler)

0900 – Ethnology Museum and lunch with Dr. Nam and epidemiologist and Hung.



1200 – Lunch Dr. Nam and wife Hung

1845 – Working dinner with Dr. Dave Dennis of CDC and Dr. Michael Iademarco of HHS in evening

- Discussed current projects
- MoH infrastructure and current relationships
- Recent changes to various processes i.e. transportation of samples
- Etc.

**Sunday – September 16, 2007** – Dr. Gubler and Bobbie depart

**Monday – September 17, 2007** – Ginger Hendee met with Thang and discussed changes to proposal and budget revisions to reflect new request.  
(no seats available for return flight for Ginger Hendee, she remains in country until Tuesday)

**Tuesday – September 18, 2007** – Ginger Hendee departs

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## **CONTACTS:**

### Ministry of Health (MoH):

***Dr. Nguyen Thi Kim TIEN;***

Vice Minister of Health

*(did not meet w/her; friend of Dr. Gubler)*

***Trinh Quan HUAN***

Vice Minister of Health for Preventive Medicine *(friend of Dr. Gubler)*

***Vu Sinh NAM PhD. Associate Professor*** *(wife is Hung and friend of Dr. Gubler)*

Dept of Preventive Medicine Dept of MOH

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***Le Thi Quynh MAI, M.D. (friend of Dr. Gubler)***

Virologist, NIHE  
Head, Influenza Laboratory, NIHE

***THANG (Truong Uyen Ninh's son)***

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**Bach Mai Hospital:**

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Home Tel: 04 835 2172

***Dr. Trinh Thi Ngoc, PhD (met her with the Director)***

Vice Head of Infectious Department of Bach Mai Hospital  
Office: 78 Giai Phong Road Hanoi City  
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[trinhtngoc@yahoo.com](mailto:trinhtngoc@yahoo.com)  
Home: No 11/56 -192 Lane; Le Trong Tan – Home Tele: 84 4 5 650 587

***Pham Thi Thanh THUY, MD***

Rickettsia Specialist at Bach Mai Hospital; met her after mtg w/Dir.

***Do Duy YUONG, MD***

Pham Thi Thanh's colleague – he works on HIV

Lang Son Province:

*(We met the female Dr. first and then the director in upstairs conference room w/restroom at end of conference room; had HIV, general infection, etc wards on various floors)*

***Dr. Nguyen Xuan Truong***

Director, Centre for Preventive Medicine of Lang Son Province

Office: No 50 Tran Hung Dao Street

Lang Son Town *(this is where we took pic of Uncle Ho & lab was barely used)*

Tel: (025) 812 662 Home: (025) 811 629

***Dr. Nguyen Minh Diep (female at the meeting)***

Director, Laboratory

Lang Son Hospital

Vietnam National Institute of Maritime Medicine in Haiphong:

***Nguyen Truong Son, MD, PhD (hyperbaric chamber, etc.)***

Director of Institute (VNIMM)

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170 Ngoc Khanh St. Hanoi, Vietnam

CDD @ embassy:

***David Dennis, M.D. MPH***

Centers for Disease Control

East-West Center and others:

- *Phil Easterman*

- *Robert Robertson at NIH? PBIV project in South w/ typhus vaccine in children*
  - *Bruce Wilcox - UH*
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### **Collection Sites:**

1. Bach Mai Hospital  
Add: Giai Phong St., Dong Da Dist. Hanoi  
Tel: (84-4) 869 3731 / 869 3732
2. Lang San – Provincial Hospital
3. Viet Tiep Friendship Hospital  
Add: Nha Thuong street; Haiphong  
Tel: 700 436
4. Hoa binh – District Hospital (Preventive Medicine)
5. Cao Bang Polyclinical Hospital (Preventive Medicine & District Hospital)  
Address: Dong Khe Road, Hop Giang Ward, Cao Bang Town, Cao Bang Province  
Tel: 026-8558100

## Appendix VII: Microsphere Immunofluorescent Assay (MIA) results, Task 2.

TABLE 1: Cohorts used for comparison of anti-dengue virus IgG antibodies using MIA, IgG ELISA and PRNT											
Cohorts	Blood Draw Year	Serum Specimens	MIA		PRNT			IgG ELISA			% ELISA Congruity*
			Positive	Negative	Positive	Negative	% PRNT Congruity*	Positive	Negative	Equivocal	
Yap 2007	2007	69	64	5	64	5	100	49	9	4	85
French Polynesian 2007	2007	14	12	2	12	2	100	9	2	3	79
Oahu, Hawaii 2001	2003/2004	13	12	1	12	1	100	12	1	0	100
Oahu, Hawaii 1943	2005	7	4	3	4	3	100	2	4	1	71
Maui, Hawaii 2001	2008	15	14	1	14	1	100	9	4	2	67
Hawaii, Hawaii 2007	2007	73	0	73	0	73	100	0	73	0	100
Palau, 2008	2008	18	14	4	14	4	100	13	4	1	94
American Samoa, 2008	2008	19	19	0	19	0	100	8	8	0	50
Healthy Individuals, HI	2008	54	13	41	13	41	100	1	28	0	93
Proficiency Panel	NA	10	2	8	2	8	100	3	8	0	100
Total	NA	292	137	121	137	121	100	106	141	11	89

MIA, Microsphere-based Immunoassay; PRNT, Plaque Reduction Neutralization Test; \*percentage of serum specimens if MIA positive or negative were also PRNT positive or negative; \*\*percentage of serum specimens if PanBio IgG ELISA positive, negative or equivocal were also PRNT positive or negative

TABLE 2: Comparison of anti-DENV IgG and IgM antibodies and NS1 antigen using MIA, IgG PanBio ELISA and IgM PanBio ELISA					
		IgG MIA	IgM MIA	NS1 MIA	NS1 Platelia
	Serum Specimens	Positive	Positive	Positive	Positive
Total Samples	123	90/123 (73%)	88/123 (72%)	105/123 (85%)	54/110 (49%)
IgM Negatives	35	24/35 (69%)	0/35 (0%)	17/30 (57%)	10/29 (34%)
IgG Negatives	33	0/33 (0%)	22/33 (67%)	25/33 (78%)	24/33 (73%)
NS1 Negatives	18	9/18 (50%)	8/18 (44%)	0/18 (0%)	7/18 (39%)

MIA, Microsphere-based Immunoassay; \*percentage of serum specimens if PanBio IgG ELISA positive, negative or equivocal were also MIA positive or negative.